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STORY OF
THE TUBERCULIN TEST

From an Epidemiologic Viewpoint

by

PHYLLIS Q. EDWARDS and LYDIA B. EDWARDS

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FOREWORD

The views set forth in a fresh and breezy style in the following supplement furnish a much needed analysis in a time of renewed concern over the specificity of the tuberculin reaction. As the authors declare, tuberculin is again on trial. Once, when mass surveys disclosed a conspicuous inability of the tuberculin test to elicit reactions in certain large population groups with calcified pulmonary lesions that were accepted at the time as residues of old tuberculosis, the value of the test as a diagnostic means was perforce heavily discounted. The discovery, however, of the relation of these lesions to Histoplasma infection rather than tuberculosis restored faith in the test and enhanced its reputation for specificity.

Now a new problem is apparent, of opposite implication. Whereas, before, tuberculin failed to identify certain supposedly tuberculous lesions as tuberculous, now it seems to put the label of tuberculous infection on many persons who have not been infected with tubercle bacilli. To the authors this is a harder problem than the first one, but they point to a precedent that may be useful in solving it, the "no-lesion reactor" in cattle. The explanation of the reaction may be the same in the two instances, viz., the capacity of tuberculin to elicit "cross reactions" in mammalian organisms sensitized to some degree with antigenic components chemically related to but not identical with those of tubercle bacilli.

It has long been known that acid-fast bacilli other than true tubercle bacilli infect men and domestic animals. The differentiation of these organisms from tubercle bacilli has not always been easy. The difficulty has been relatively small in the case of leprosy and Johne's disease of cattle, even though much remains to be learned of the bacteriology of these infections. The problem is less simple, however, in the case of other acid-fast microorganisms. In recent years there has been, in the pithy phrase of the authors, a "torrent of newly recognized mycobacteria." It is still difficult to give these "atypical" microbes a name; and some investigators prefer to set them apart simply as "anonymous," using a few identifying terms such as photochromogens, skotochromogens, nonchromogens, "yellow bacilli," "Battey strains," and so forth, as temporary labels pending the acquisition of enough facts for better identification. The microorganisms differ culturally in many ways from true tubercle bacilli, but they are not all harmless saprophytes, and they have the capacity, on inoculation, to confer on animals some degree of sensitivity to tuberculin. The fact that an appreciable number of the strains are truly pathogenic, setting up granulomatous lesions with some similarity to tuberculosis, compounds the problem. Most of the bacteria are believed to come from extracorporeal reservoirs in nature, but a few investigators have gone so far as to suggest that some of them may be mutants of true tubercle bacilli, shed from the parent stock under the influence of chemotherapy.

For years it has been accepted as a fact that the tuberculin reaction varies widely in intensity among persons known to have been infected with tubercle bacilli, whether they carry the results of the infection as active disease or as healed lesions that can be detected and identified by other diagnostic tests. Intense reactions are often given by apparently healthy persons who have been exposed over short or long periods to known infectious patients. In these cases there is little tendency to doubt the specificity of the reaction even if no close correlation holds between the strength of reaction and the type and extent of lesion, but there has been real doubt if, while telling the truth, the test tells the whole truth, i.e., whether it detects all of the persons who have been infected, including those developing but slight hypersensitiveness to tuberculin. To make sure of the latter, it has been standard procedure to administer the test in two strengths, the first, with the active component in high dilution, to bring to light high sensitivities with safety; and the second, much more concentrated, to detect persons with low sensitivity.

It has always been granted that the second of these purposes was achieved at the expense of including some false "nonspecific" reactions. To avoid these as far as possible, the most potent chemical procedures have been used to refine tuberculin, eliminating inert material and irritating products of bacterial metabolism not specific for tubercle bacilli.

In the old days, the majority of those who reacted to tuberculin at all reacted to the first strength. This was in a period when tuberculosis was a much more common disease than it is today. In more recent years the trend has been reversed. In many surveys a far higher proportion of those inoculated with tuberculin do not react to the standard first strength, and react only weakly to the second. In the old view it was simply assumed that the weak second-test-strength reactions were merely evidence of low sensitivity to products of the tubercle bacilli. More recently, however, Carroll Palmer and his associates have thrown serious doubt on this assumption. They have suggested, with increasing evidence, that some of these weak reactions—and indeed the majority in certain parts of the country—are due not to infection with tubercle bacilli, but to infections with some of the numerous mycobacteria now coming to light. The particular virtue of these studies, which are analyzed at length in the following pages, is their recognition of the possibilities of what Phyllis Edwards and Lydia Edwards call a "fractionation" of the tuberculin test.

Fractionation of tuberculin is now an old story, but "fractionation" of the test is new. Fractionation of the "raw" tuberculin that comes from the growth of tubercle bacilli, yielding products of varying capacity to elicit reaction in tuberculosis, has been accomplished by Seibert and her associates with a high degree of precision. Now we are learning that products from the newly discovered mycobacteria, prepared by exactly the same procedures as those employed in the isolation of the potent chemical constituents of true tubercle bacilli, are showing promise in identifying other infecting agents, in spite of "cross reactions." There is some evidence that by keeping the test dose with tuberculin small, and reading a reaction as positive only at a level of intensity somewhat higher than that formerly accepted, many of the reactions to tuberculin that are not due to true tuberculous infection can be counted out. This would be great progress of itself, but the evidence goes further, suggesting that the various tuberculin-like preparations made from the suspected mycobacteria by Lewis Affronti with the Seibert techniques may then be added to the test procedure in such a way as to identify the offending microorganism on the basis of maximal sensitivity. Certainly it does not seem far-fetched to compare, as the authors have done, the frequency curves of distribution of reaction, by dosage and intensity, with the curves and peaks visible in electrophoretic analysis in the fractionation of tuberculin.

This, as in the case of other efforts in the past, may turn out to be an oversimplification. On the other hand, the evidence set forth by Phyllis Edwards and Lydia Edwards is impressive as an indication of possibilities. There appears to be substantial ground for trust that the suggested ways of using tuberculin and analogous products from "atypical" mycobacteria will meet success like that attained when the tuberculin test was supplemented with the histoplasmin test. Certainly the data presented warrant watching the forthcoming continuation with much hope as well as interest.

Esmond R. Long
Pedlar Mills, Virginia

October 15, 1959

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CHAPTER I

THE BEGINNINGS

A curative agent against tuberculosis was announced by Robert Koch at the Tenth International Congress of Medicine held in Berlin on August 4, 1890 (1). The agent was described simply as a "brownish transparent fluid" which, he said, protected against tuberculosis in guinea pigs and also cured established disease. Further details were to await results obtained in tuberculous patients currently under treatment. Three months later, on November 13, 1890 (2), Koch published his findings: the fluid had a specific healing effect on tuberculous processes of all kinds in human beings. Rapid healing took place in the milder cases; slow but progressive improvement in the more severe.

His report was immediately hailed by both the medical and lay press, as Robert Koch was the scientist who, eight years earlier, had isolated and cultured the microbe he called the tubercle bacillus, and with it had reproduced tuberculous disease in animals. On November 22, 1890, *The Lancet* of London commented editorially (3):

> The publication by Professor Koch of an account of the method of treating tubercular disease which, as the result of years of patient research, he has discovered, may be taken as evidence that he himself is satisfied of its efficacy. No doubt this publication has been in a sense forced on him by the extraordinary anxiety manifested on all sides to learn something tangible concerning a remedy about which the greatest possible expectation has been raised. From one point of view, therefore, this early publication is to be deprecated, since it does not contain any records of cases, or any account of the lines pursued by the discoverer in the production of the remedy, and no exception can be taken to a postponement of final judgment until all these facts are before the profession. Nevertheless there are few men more worthy of confidence than Professor Koch, whose whole record is that of the patient, earnest seeker after truth, and who has already done so much to widen our conceptions of disease. That this is the general opinion is shown by the reception given to the first intimations by him that he was within reach of succeeding in his search for an antidote to the tubercular virus. Such a statement from many another source would have been received with more than incredulity, but this announcement, when made at the International Medical Congress, was welcomed as "glad tidings of great joy," for it was generally felt that Robert Koch was a man who seldom made a statement which he could not justify, and one who, in this particular instance, must have felt the tremendous issues depending on his utterances.

Koch's treatment consisted of daily doses of the fluid given by subcutaneous injection between the scapulae, starting with 0.01 ml. and rapidly increasing the dose.[1] No reaction appeared at the site of the injection, but in the tuberculous patient the temperature began to rise after about four to five hours, reaching a peak of 102° to 104°F. in ten to twelve hours, and then subsiding. This febrile reaction was usually accompanied by vomiting, rigors, and other constitutional symptoms. The fluid was believed to promote healing through its specific action, not on the tubercle bacilli, but on the "living tuberculous tissue" in which the bacilli were enmeshed. In the nontuberculous person, the only reaction to injection of the fluid was slight pains in the limbs, a sense of transient fatigue, and a small rise in temperature, if any.

In the following months there was heated controversy on both sides of the Atlantic as to whether Koch was justified in keeping secret the nature of his fluid. On December 13, 1890, however, Sir Joseph Lister of England (5) went on record in support of Koch:

> Koch, I believe, would not have published his method at all, had it not been for great pressure brought to bear upon him, until he could have produced it in a form capable of being revealed in every detail. . . . And I must say that the carping against Koch on account of what is spoken of as a "secret remedy" can only proceed from absolute ignorance of the beautiful character of the man.

A week later, *The Journal of the American Medical Association* (6) also expressed belief in Koch's integrity:

[1] *The Lancet* for December 6, 1890 (4) noted "That five grammes of the fluid, sufficient for 5000 injections, should cost 25 marks is not surprising, and, indeed, it shows that it is, *quoad* its alleged effects, perhaps the cheapest drug ever placed on the market."

> It would seem that any one who had devoted eight years to the solution of a problem of the utmost importance to science and humanity ought, at least, to be accorded the meagre privilege of dictating the manner of its publication. Dr. Koch has shown no disposition to profit directly by his discoveries, and it would seem that if he withholds it for a time it is probably for a good reason, and with his simple statement the profession ought for the present to be satisfied.

In January, 1891, Koch (7) disclosed that the fluid was a broth culture filtrate of tubercle bacilli to which he gave the name tuberculin. Unhappily, his claims for the protective and curative properties of tuberculin were soon to be challenged, and criticism became outspoken. For example, in April, 1891, the Senior Physician to St. Thomas's Hospital, John Syer Bristowe (8), in an address on the Koch method of treatment for tuberculosis, said that ". . . many persons— . . . also in our own profession—seem fairly to have lost their heads. Scrofulous joints and lupus have been cured . . ., the only grounds for the allegations of these successful results being an unreasoning faith in Koch's infallibility, . . ." And in December, when the report of the Tuberculin Treatment Subcommittee of the Hospital for Consumption, Brompton, was issued, the *British Medical Journal* (9) said of the report: "On the whole, perhaps no more damning piece of evidence has yet appeared." Koch's claims for the use of tuberculin as a cure for tuberculosis had, indeed, proved ill-founded: he had tried to fit a square peg into a round hole.

For tuberculin as a diagnostic agent, however, the story was entirely different: the peg fit the hole. Subcutaneous inoculation of tuberculin in the tuberculous subject led to a sharp rise in temperature which lasted for several hours; but it had little or no effect on the nontuberculous. Koch's observations on this marked difference in response to tuberculin between the tuberculous and the nontuberculous subject proved to be sound, and laid the foundation for one of the most valuable and most widely used diagnostic tests in both veterinary and human medicine and in the field of public health. Moreover, the method of preparing Old Tuberculin, as Koch's fluid is known today, is still essentially the same as Koch described it in 1891.

The use of tuberculin as a diagnostic agent led to the development of new methods of administration which eliminated the generalized reaction and caused, instead, only a localized reaction at the injection site in the skin. In general, the new testing procedures involved the administration of tuberculin onto or into the skin rather than into the subcutaneous tissues. By 1910 the roster of methods included a cutaneous test (von Pirquet), a percutaneous test (Moro), a conjunctival test (Calmette), a modification of Koch's original subcutaneous test (Hamburger), an intracutaneous test (Mantoux), and other variations. Each was purported to possess distinct advantages over the others; and each, in its turn, inspired loyal proponents. Three methods have survived: the Pirquet cutaneous test, the Moro patch (percutaneous) test, and the Mantoux intracutaneous test.

The intracutaneous test is by all odds the most vigorous survivor. It is the most widely used, and it is also the most precise. Its popularity is surprising inasmuch as it involves precision of administration; and when a procedure is put into the hands of literally thousands of skilled and unskilled persons all over the world, precision is often sacrificed for more practical considerations, such as simplicity and ease of administration. The cutaneous and the patch tests require no syringes, no needles, and no dilutions. The intracutaneous test, on the other hand, requires the use of a calibrated syringe and a needle for the injection of a carefully measured amount of diluted tuberculin into the most superficial layer of the skin. The reaction appears after twenty-four hours or so as a red, rounded swelling in the skin which persists for a few days and then disappears.

Reactions to the intracutaneous test generally were not, until recently, measured carefully but were commonly interpreted simply as "positive" or "negative," as judged subjectively by the eye and not by a rule. This is also surprising. If precision in dosage and technique of administration was deemed significant why, then, was so little importance attached to precision in measurement of the response?

Indeed, the story of the tuberculin test is punctuated with the unexpected, with periods of high hopes broken by periods of doubt and disillusion about its usefulness as a diagnostic test.

Shortly after its introduction, the tuberculin test was recognized as a potent weapon in the

fight against bovine tuberculosis. Enthusiasm ran high. Yet the development of the test for widespread use in cattle was marked by periods of discontent and criticism which, at times, were stormy. Nevertheless, it was this discontent and criticism that triggered research and eventually resulted in practical techniques for distinguishing between tuberculin reactions caused by virulent tubercle bacilli and reactions caused by other, usually harmless, organisms.

The medical profession, meanwhile, found the tuberculin test of limited usefulness because practically everybody in the early part of this century reacted to tuberculin. In fact, for many years the test was primarily a diagnostic aid in clinical practice; and a reaction to any dose of tuberculin, even a very large one, was considered to be indicative of tuberculous infection. Indeed, as Krause wrote in 1916 (10), "... a positive cutaneous test means infection or the presence of tubercle, ..."

Doubts about the specificity of the tuberculin test in humans first arose in the United States during the 1930's and led to spirited controversies when pulmonary calcifications were discovered in large numbers of persons who did not react to tuberculin. At that time pulmonary calcification was regarded as a sure sign of healed tuberculosis. A negative tuberculin test in conjunction with pulmonary calcification could only mean, according to some, that tuberculin sensitivity disappeared with "biological healing" of the tuberculous focus. Others recognized the possibility that pulmonary calcification might not always be caused by tuberculosis; and it was this hypothesis that led to the investigations which showed that infection with the fungus *Histoplasma capsulatum* not only is a common cause of pulmonary calcification, but it is also highly prevalent in the particular areas of the country where the tuberculin test had seemingly failed.

Today, faith in the tuberculin test is again vacillating as it becomes overwhelmingly evident that the old-fashioned garden variety of tubercle bacillus is not the only cause of tuberculin sensitivity in human beings. Some of the tuberculin sensitivity—and in some regions a very high proportion of the tuberculin sensitivity—apparently is caused by generally harmless infection with "first cousins" of the tubercle bacillus. Thus a positive reaction can no longer be taken as a sure sign of tuberculous infection. However, the progress already made toward the solution of this problem indicates that the present period of apostasy, like the earlier ones, soon will become only another incident in a future story of the tuberculin test which will probably deal with its role in the eradication of tuberculosis as a public health problem.

CHAPTER II

THE CASE OF THE NO-LESION REACTOR IN CATTLE

The potentialities of Koch's tuberculin as a diagnostic tool for identifying tuberculous cattle were immediately recognized by veterinarians. According to Myers (11), it was in January, 1891, only five months after Koch's paper was read in Berlin, that Professor Guttman of the Veterinary Institute of Dorpat, Russia, used the product with cattle for diagnostic purposes and achieved the distinction of being the first in the world to do so. Myers states that in the same year Bernhard Bang of Denmark became convinced, after extensive experiments, of the diagnostic value of tuberculin when used with cattle. The following year, according to Marshall (12), Professor Eber of Berlin reported that, of 134 cattle reacting to tuberculin, 85 per cent showed lesions at autopsy; and 89 per cent of 113 with no reaction to the test were found to have no lesions on autopsy.

Leonard Pearson, an American veterinarian working in Koch's laboratory at the time tuberculin was announced, undoubtedly had full knowledge of these investigations. Following his return to this country, Pearson, in Pennsylvania in March, 1892, gave a dramatic demonstration of the value of testing cattle with tuberculin. Using Old Tuberculin brought with him from Berlin, he tested the Gillingham herd of 79 animals and found 30 reactors.[2] These 30 animals were slaughtered and post-mortem examination, witnessed by a skeptical group of veterinarians and cattle owners, showed the presence of tuberculous lesions in all of them. Events which led up to this historic occasion were described by Pearson (14), who at that time was only twenty-three years old and held the title of Assistant Professor of the Theory and Practice of Veterinary Medicine in the University of Pennsylvania:

> I was requested by Mr. Joseph E. Gillingham, several weeks ago, to examine a cow in his herd which did not seem to be doing well, and I found it suffering from tuberculosis. The animal was killed the next day, and the diagnosis was confirmed by the post-mortem examination. Thereupon I made a careful physical examination of the entire herd, and found five cows affected with the same disease, all of which were promptly destroyed. . . . This caused me to suspect that the disease might be more prevalent than we heretofore had reason to suppose, and with the object of detecting the affected animals it was decided to use tuberculin as a diagnostic agent. It is well known that tuberculosis in cattle is exceedingly difficult to diagnosticate, and that there are no physical signs by means of which we can recognize any but well-advanced stages of the disease. It is for this reason that tuberculin is so valuable as a diagnostic agent. The subcutaneous injection of a small quantity of tuberculin is followed by elevation of the temperature of a tuberculous animal, but not otherwise.
>
> In all, seventy-nine of Mr. Gillingham's cattle have been tested with tuberculin, and of these thirty have reacted in a manner that has been interpreted as indicating the existence of tuberculosis.
>
> Of these, two have been killed, and were found to be diseased. After discovering how prevalent the disease probably was in the herd, the question of the disposal of the animals became the all-important one. Three courses were open: The animals might have been kept, as they were all apparently in good condition and appeared well, and many were yielding large quantities of milk; or they might have been sold, for they seemed to be in perfect health, and would undoubtedly have brought good prices; or, lastly, they could be killed, and thus the danger of contamination of the other animals and of the milk be removed. Mr. Gillingham did not, for a moment, consider any but the last method of disposing of the animals. As soon as tuberculosis was discovered, he ordered the affected cows destroyed, and the milk from animals known to have tuberculosis has never been sold or used.

Following Pearson's demonstration, which gave undeniable proof of the value of the tuberculin

[2] Mr. Gillingham, a Trustee of the University of Pennsylvania, was said to have had "an exceptionally fine" herd of Jersey cattle, ". . . selected with the greatest judgment, and . . . maintained under the best hygienic conditions. An ordinary physical examination would have failed to detect signs of disease. As a part of a plan to employ every measure available for determining the soundness of the cattle, it was resolved to subject them to a diagnostic test that has recently been the subject of a good deal of heated discussion." (13)

test as a means of earmarking diseased cattle for disposal, campaigns aimed at eradication of bovine tuberculosis in this country soon were organized. With periodic testing and the prompt removal of all reactors, the numbers of tuberculous herds decreased fairly rapidly. Yet, as the testing of cattle became more widespread and was extended beyond those herds suspected of having tuberculosis, doubts were raised as to the specificity of the tuberculin test. Some of the cattle that were slaughtered and subjected to post-mortem examination did not show signs of tuberculous infection even though they had reacted to tuberculin. As the frequency of tuberculous animals decreased over the years, the proportion of "no-lesion" reactors gradually increased. Hastings and his co-workers (15) reported that in Wisconsin, for example, 48,000 cattle were tested during the year 1909. About 3 per cent reacted to the tuberculin test and more than 17 per cent of those failed to show lesions. The next year, 1910, the number of tests in Wisconsin rose to 207,000. While the frequency of reactors was again about 3 per cent, the proportion of no-lesion animals among the reactors had risen to 22 per cent.

As Hastings (16) wrote in 1914: "The occurrence of no-lesion animals did not attract attention in this state [Wisconsin] as long as the testing was restricted, in large part, to such [suspected] herds but with the more general application of the test the no-lesion animals became more numerous."

Although some workers were claiming that the cause of reactions in no-lesion cattle could be attributed to variations in technique of administering the test, Hastings showed that in Wisconsin, at least, the percentage of no-lesion reactors was no greater among cattle tested by nonprofessionals than among those tested by registered veterinarians. He did, however, find an important clue by a study of the occurrence of no-lesion animals in relation to the extent of tuberculous disease in the herd, for it had been noted that many of the no-lesion reactors were appearing in herds with only a few tuberculous animals.

Data for 5,726 tuberculin-reacting animals slaughtered in 1910–1912 are reproduced in table 1 from Hastings' report in 1914 (16). Of those herds in which only one animal reacted to tuberculin, 44 per cent of the slaughtered animals were found at post-mortem examination to have no tuberculous lesions; from herds with 3 or 4 reactors, 27 per cent had no lesions; while from herds having 10 or more reactors, only 7 per cent had no lesions. Thus, the frequency of no-lesion reactors was inversely related to the number of tuberculous animals in the herd, those with the lowest frequency of demonstrable tuberculosis having the highest proportion of no-lesion reactors. Although Hastings concluded his report in 1914 with the statement that most of the no-lesion reactors probably had tuberculous lesions not detected on routine autopsies, by 1924 he believed that the accumulated evidence pointed not to tuberculosis but rather to sensitization to tuberculin by some other organism or organisms (17).

TABLE 1

THE RELATION BETWEEN THE FREQUENCY OF NO-LESION ANIMALS AND THE NUMBER OF ANIMALS REMOVED FROM THE HERD

(Reproduced with permission from Hastings, Bull. Univ. Wisconsin Agr. Exper. Sta., 1914, No. 243)

Number of Animals Removed from Herd	Number of Herds from which Animals Were Removed	Number of Animals Killed	No-Lesion Animals	
			Number	Percentage
1	1,063	1,063	468	44.0
2	346	692	248	35.8
3 or 4	110	699	188	26.9
5 to 9	207	1,167	135	13.1
10 or more	120	2,105	145	6.8
Total	1,846	5,726	1,184	20.6

In Pennsylvania, the reactor rate among cattle had been reduced approximately 50 per cent between 1895 and 1920, yet veterinarians were becoming alarmed by what Bruner (18) called the inefficiency of the tuberculin test. For example, from herds tested under the accredited plan in 1918–1920, 9 per cent of 1,600 tuberculin-positive cattle had no lesions, while from 12 herds owned by public institutions 29 per cent were no-lesion reactors. Moreover, the proportion of no-lesion reactors had been increasing rather than decreasing during the five-year period ending in 1920, despite the use of tuberculin prepared in a uniform manner, more accurate autopsy reports, and a steady reduction in the frequency of tuberculous animals. This, Bruner wrote, indi-

cated that some animals that are not tuberculous are sensitive to tuberculin and at times give typical reactions.

During the following decade, according to Hagan (19), the percentage of no-lesion reactors in the entire country rose from less than 8 per cent in 1920 to more than 11 per cent in 1930, although the tuberculosis rate in that period had dropped considerably. But the proportion of no-lesion reactors was by no means constant in all parts of the country. Of the tuberculin reactors in Utah, for instance, Daines and Austin (20) reported that 32 per cent showed no lesions at autopsy; while, in New York State, Hagan (19) reported that 17 per cent of the reactors in 1930 were no-lesion animals. Hagan also pointed out that one would expect the decrease of bovine tuberculosis to be accompanied by a similar decrease in the percentage of no-lesion reactors if the slaughtered cattle were, in fact, animals with incipient tuberculosis, as some still believed. On the other hand, if the no-lesion reactors did not represent some form of tuberculosis, their proportion could be expected to rise as the tuberculosis rates fell. And it did, accompanied by the cries of farmers outraged at the slaughter of nontuberculous animals.

CHAPTER III

CAUSES OF NONSPECIFIC TUBERCULIN SENSITIVITY IN CATTLE

Widespread occurrence of so-called saprophytic acid-fast bacteria suggested to Crawford (21) of the Bureau of Animal Industry Experiment Station at Bethesda, Maryland, that such organisms might at times be associated with skin lesions in cattle. Using guinea pigs as experimental animals, he found that sensitization to both mammalian (human or bovine) and avian tuberculin could be induced by injection of cultures of a variety of acid-fast saprophytes, including the Friedmann turtle bacillus, the hog-skin bacillus, the grass bacillus, and others. On the whole, reactions were inconstant, but in a few instances they could be differentiated from specific tuberculin reactions only by their size and their tendency toward earlier resolution. In a later report (1927) on studies of guinea pigs inoculated with human, bovine, BCG, and four avian strains of tubercle bacilli, and given comparative (simultaneous) intracutaneous tests with antigens made from these organisms, Crawford (22) found that reactions to the homologous antigen[3] were invariably well marked and constant, while reactions to heterologous antigens tended to be less marked, more variable in size, and inconstant.

A number of studies followed, clearly demonstrating that sensitization to tuberculin can result from infection with various acid-fast strains. Hagan and Zeissig (23), for example, showed in 1929 that guinea pigs infected with avian tubercle bacilli or *M. johnei*[4] will react to intracutaneous injections of either avian tuberculin or johnin (the antigen prepared from the Johne bacillus); and, when applied to cattle and other animals naturally infected with Johne's disease, avian tuberculin and johnin apparently give identical results. Later, Frey and Hagan (24) demonstrated cross-reactions within groups of various acid-fast bacteria cultured from soil samples collected all over the United States. Organisms from any one of three broad groups showed a closer relation to other members of the same group than to organisms of the other groups, judging by sensitization studies of guinea pigs. Hastings, Beach, and Thompson (25) reported sensitizing young cattle to tuberculin by inoculation of several different strains of nonpathogenic acid-fast organisms; even though in most cases the sensitivity was evanescent, the writers concluded that ". . . a positive response in cattle to tuberculin is not absolute proof of infection with tubercle bacilli." Seibert and Morley (26) found, in guinea pigs infected with virulent and avirulent strains of human tubercle bacilli, that reactions could invariably be obtained with large doses of protein antigens prepared from avian and timothy grass bacilli. This, they believed, was evidence that the animal body is able to make a quantitative distinction between the proteins from different types of acid-fast bacilli, but not, apparently, between proteins from different strains of the same type.

In reviewing the no-lesion reactor problem in 1931, Hagan (19) pointed out that tuberculin as a diagnostic agent is not infallible, despite Calmette's insistent claim that a positive reaction always means that the animal has a tuberculous infection, or at least is harboring tubercle bacilli in some parts of its body. This might be true, Hagan admitted; but it was a statement that could not be proved, and considerable evidence had accumulated indicating that it was not always true. In the past, he continued, it had been generally assumed that the no-lesion reactors were tuberculous in a slight degree—the tuberculous lesions simply escaped detection. He wrote:

> When the tuberculosis rate in cattle was high this explanation served, but recently

[3] The homologous antigen is prepared from the same organism as the one used to infect the animal. The reaction is therefore specific. A heterologous antigen, which is one prepared from a different organism than the infecting agent, causes a nonspecific or cross reaction (if any).

[4] Named for Professor Johne, of the Dresden Veterinary High School, who in 1895 discovered this acid-fast organism as the cause of a form of chronic dysentery of cattle. The disease was called "paratuberculosis" by Bang, but later renamed Johne's disease because "paratuberculosis" was thought to be misleading. Johne's disease is widespread in many parts of the world, including India, Western Europe, and the United States (23).

since a determined effort has been made to eliminate the infection, too many instances have occurred in which the reacting animals were almost certainly not infected with tuberculosis for us to retain the old idea as a wholly adequate explanation.

The probable cause of the majority of the no-lesion reactors, he believed, was sensitization by nonpathogenic acid-fast organisms which are abundant in nature. That such organisms could invade through the skin and alimentary tract had already been shown, but not that they could cause tuberculin sensitivity under natural conditions. Subsequent work by numerous investigators, prominent among whom was Plum (27) in Denmark, showed conclusively that under natural conditions infection with avian tubercle bacilli could certainly account for some, though by no means all, of the no-lesion reactors.

Green (28) of the Weybridge Laboratories, England, in 1946 published his classic chart of "Specificity Factors" for purified protein derivatives (PPDs) prepared from several acid-fast organisms, including BCG, *M. johnei*, and the nonpathogenic *M. phlei*. The specificity factor was defined as the number of weight units of heterologous PPD required to elicit the same intensity of skin reaction as one unit of homologous PPD. Using 240 guinea pigs divided into eight groups, with each group sensitized by one of the six organisms being studied, he reported the specificity relations among the corresponding PPD preparations shown in table 2.

Green's animals were sensitized by inoculation of live organisms in the case of the human, bovine, BCG, and avian bacilli; and with dried organisms in oily suspension for Johne's bacillus and *M. phlei*, which are not infective for guinea pigs. As shown in the table, cross reactions were obtained with all six PPDs, with only small differences in the specificity of human, BCG, and bovine PPDs. Phlei PPD differed most widely from the mammalian tuberculins: 150 units were required to elicit a reaction of the same size as 1 unit of either human, BCG, or bovine PPD. And twice as much avian tuberculin was required to elicit a reaction of the same size in bovine-sensitized animals as in animals sensitized with human bacilli.

Although several decades ago most veterinarians abandoned the traditional belief that tuberculin sensitivity is necessarily specific for tuberculous infection, not every one could accept

TABLE 2

SPECIFICITY FACTORS* OF PPD ANTIGENS PREPARED FROM DIFFERENT ORGANISMS, AND USED IN TESTING GUINEA PIGS SENSITIZED BY INFECTION WITH THE HOMOLOGOUS AND HETEROLOGOUS ORGANISMS

(*Adapted from Green, Vet. J., 1946, 102, 267*)

Type of Sensitization	Type of PPD Antigen					
	Human	Bovine	BCG	Johne	Avian	Phlei
Human	1	½	2	30	20	150
Bovine	1	1	2	30	40	150
BCG	1	½	1	30	20	150
Johne	10	10	10	1	3	50
Avian	20	40	40	3	1	100
Phlei	150	150	150	50	100	1

* The number of weight units of heterologous PPD required to elicit the same intensity of skin reaction as one unit of homologous PPD.

the obvious evidence. Plum (27) remarked in 1937 that: "A few veterinary surgeons have had difficulty in subscribing to the idea, that reaction to bovine tuberculin needs not necessarily mean that the animal is suffering from bovine tuberculosis, . . ." Today it is known that infection with avian tubercle bacilli is the cause of some of the nonspecific reactions; it is also known that some are caused by human tubercle bacilli, by Johne's bacillus, and several others. But, according to Paterson (29) of the Weybridge Laboratories, the majority of the no-lesion reactors in cattle must still be ascribed to unidentified causes.

As to the prevalence and distribution of nonspecific reactors, Paterson (30) stated that only very general estimates are available, and those for only a few countries or sections of countries. In the United States, for example, the proportion of nonspecific reactors among all positive reactors may be well over 50 per cent in some areas where the total proportion of all positive reactors to tuberculin is below 1 per cent. In England, a sample of 100 tuberculosis-free herds showed about 17 per cent with nonspecific reactions; and in Denmark approximately 3 per cent of some 2,000 reactors slaughtered during the eradication campaign had no visible lesions. Because of the generally scanty knowledge of the prevalence and distribution of nonspecific sensitivity throughout much of the world, Paterson wrote that:

> . . . a valuable study from the epizootiological aspect would be to carry out standardized tests in different world areas on randomly selected tuberculosis-free herds of comparable productive and geographical status. Such investigations would give little information on causes of sensitization, but would permit quantitative comparison of reactivity in a way at present impossible, and eliminate much subjective opinion on the incidence of non-specific sensitization.

Thus, although the availability of precise data on the causes and prevalence of nonspecific tuberculin sensitivity in cattle leaves much to be desired, its existence has long been recognized by most of the veterinary profession, and a practical way of handling the problem was developed some years ago. Nonspecific reactions can be distinguished with a fair degree of accuracy from specific reactions through knowledge of the history of the herd, combined with repeated testing or the so-called comparative test with avian and mammalian tuberculins.

CHAPTER IV

QUESTIONS OF DOSAGE AND PURIFIED TUBERCULIN

Nonspecific tuberculin sensitivity was recognized as a problem in human beings more slowly than it was in cattle, perhaps for several reasons. Obviously, the "test and slaughter" method of the veterinarians could not be used in human reactors in order to determine the presence or absence of tuberculous lesions by post-mortem examination; moreover, few dared question the credo, classic since the days of Koch, that all tuberculin reactions were caused by tuberculous infection.

Early in the present century, when more than 90 per cent of the adult population in many urban communities (and a high proportion of the children) had apparently been infected with tubercle bacilli, the tuberculin test was used chiefly in clinical practice. As the prevalence of tuberculous infection declined over the years, the epidemiologic potentialities of the tuberculin test became apparent. By the late 1920's and the early 1930's, tuberculin testing was gradually being extended to apparently healthy persons to ascertain the prevalence of infection in different age groups and in different segments of the population. The common practice of giving a series of four or five graded doses of tuberculin was impractical for large population studies, since it was not easy to get people to return repeatedly for the next stronger dose.[5] For purely practical reasons, if for none other, the question being asked at that time was: how many of the graded doses must be used in order to detect *as many reactors as possible?* It was in attempting to answer that question that the problem of nonspecific tuberculin sensitivity in human beings finally became inescapable.

After a study of more than 1,000 clinically tuberculous patients of all ages and 750 clinically nontuberculous patients, D'Arcy Hart (31) came to the conclusion in 1932 that there was no advantage in giving a dose stronger than the 1:10 dilution of Old Tuberculin. He tested both of these groups with serial tenfold dilutions, ranging from 1:10,000 to undiluted OT and found that the total number of positive reactors[6] to the 1:10 dilution was not significantly increased in either group by testing with undiluted OT. He thus concluded that ". . . a 1:10 dilution of tuberculin is the practical upper limit."

It is interesting to find in D'Arcy Hart's report a suggestion that reactions to the strongest doses of tuberculin are *not* necessarily a sign of tuberculous infection, since he recommended that glycerinated broth control solutions be used in conjunction with the strong dose tests ". . . in order to prevent confusion with delayed atypical non-specific reactions." He had found that ". . . skin reactions having all the macroscopic characteristics of the tuberculin reaction were obtainable. . ." with the control solution but, he said, their rapid development and rapid disappearance helped to distinguish them from true tuberculin reactions. He thus stressed the ability of nonspecific protein substances to produce reactions similar to the true tuberculin reaction; yet he also stated that: "Under natural conditions, at any rate in Europe, the typical response to tuberculin in man is probably only seen after tuberculous infection accompanied by the formation of histological tubercles."

[5] The dose of Old Tuberculin was reckoned by some in terms of milligrams, by others in milliliters, and by still others as a dilution factor, based in each instance on the customary assumption that 1 milliliter of OT solution represents 1,000 milligrams. Thus, for undiluted OT:

0.1 ml. = 100 mg.; 0.01 ml. = 10 mg.; 0.001 ml. = 1 mg.; et cetera.

Alternatively:

1:1 dilution = 1,000 mg. per ml. = 100 mg. per 0.1 ml. (the volume injected)

1:10 dilution = 100 mg. per ml. = 10 mg. per 0.1 ml.

1:100 dilution = 10 mg. per ml. = 1 mg. per 0.1 ml.

1:1,000 dilution = 1 mg. per ml. = 0.1 mg. per 0.1 ml.

1:10,000 dilution = 0.1 mg. per ml. = 0.01 mg. per 0.1 ml.

[6] D'Arcy Hart considered a reaction positive if either the erythema or induration measured 5 mm. or more in diameter. Most other investigators have disregarded erythema and consider a reaction positive only if the induration measures at least 5 or 6 mm. in diameter. Hence, unless otherwise specified in this review, the term "positive" or "reactor" denotes a reaction or a person with a reaction of at least 5 or 6 mm. of induration.

Despite widespread acceptance of the view reflected in D'Arcy Hart's statement, several American workers who were familiar with the problem of nonspecific (that is, nontuberculous) tuberculin sensitivity in cattle suggested that an analogous situation might exist in humans. Since it was under natural conditions that no-lesion tuberculin reactions occurred in cattle, proving beyond question that tuberculin sensitivity was *not* always a result of tuberculous infection, perhaps no-lesion reactions could also occur among humans. Following the demonstration by Hastings and associates in 1930 (25) that acid-fast bacilli recovered from no-lesion reactor cattle could, by injection, sensitize nontuberculous cattle to mammalian tuberculin, Mariette and Fenger (32) were among the first to present evidence pointing to the nonspecific character of reactions to strong doses of tuberculin in human beings.

In effect, Mariette and Fenger were asking quite a different question from the earlier one: what dose of tuberculin will identify *persons who have been infected with tuberculosis* and yet will not cause "positive" reactions in persons who have not been infected? In testing nearly 3,500 persons in Minnesota, including tuberculous patients, sanatorium employees, medical students, and rural school children, they found that 92 per cent of the sanatorium patients with clinical tuberculous disease reacted to a dose of 0.0005 mg. or less of a new type of tuberculin product called MA-100 protein (33), and also to an equivalent dose of 0.01 mg. of Dorset's Old Tuberculin. Reactions were also obtained in general population groups with MA-100 preparations of bovine, avian, and timothy grass bacilli, given in doses considered equivalent to 0.01 mg. of OT and 0.0005 mg. of MA-100 human. These results suggested that a reaction to a small dose of OT or the MA-100 protein was specific for tuberculous infection, but raised a question as to

> . . . whether the reactions obtained to 1.0 mgm. (0.001 cc) of O.T. do not represent a response to sensitization by any of the acid-fast group of bacilli rather than by the tubercle bacillus alone. . . . there is apparently a protein substance common to all acid-fast bacilli, which, if given in large enough doses will call forth a typical tuberculin reaction in just as large a proportion of the people as does Old Tuberculin. One cannot help but be struck with the similarity between this phenomenon and that of the typhoid-agglutination test. In small doses or high dilutions the agglutination test is specific in that typhoid bacilli only are agglutinated. In larger doses or low dilutions the entire typhoid group is agglutinated (32).

Further studies reported by Fenger and associates (34) of tests on 6,300 persons (tuberculous patients and school children in Minnesota) confirmed and extended their earlier observations. In 104 proved cases of tuberculosis tested with Dorset's OT and MA-100 proteins of human, bovine, avian, timothy grass, and lepra bacilli, in dilutions ranging from 0.000001 to 0.0005 mg., all of the patients reacted to 0.000025 mg. or less of the bovine, to 0.00005 mg. or less of the human, and to 0.0001 mg. or less of the avian protein. Of the 5,500 children, about 1,500 were tested with 0.0005 mg. of both the human MA-100 and timothy MA-100: the percentages of positive reactors were 15.0 and 20.0, respectively. In commenting on these findings, the authors wrote:

> This raises the question of whether man can be sensitized by the timothy-grass bacillus even though it is not pathogenic for him. But what do we mean by the phrase "is not pathogenic for him?" Do we mean that it will not produce a pathological implantation or that it will not produce progressive disease? . . . various authors, including Frey and Hagan, and Hastings, Beach and Thompson, and Daines and Austin, call attention to the fairly large percentage of "no lesions" or "skin lesion" reactors in cattle. From numerous studies these authors are of the opinion that some other mycobacterium besides that of tuberculosis is the cause of the tuberculin reaction in these cattle. Furthermore, Hastings, Beach and Thompson state, 'It was necessary at the beginning of the eradication movement to consider every reacting animal a tuberculous one. It seems proper to ask whether this policy should be continued.' Has the control of tuberculosis in man progressed far enough to ask whether all of the positive reactions to Old Tuberculin or to the protein of any acid-fast bacilli are due to infection by the *Mycobacterium tuberculosis?* May not some other mycobacterium be the cause of the tuberculin hypersensitiveness in man or in childhood at least, even though it may be only a transitory reaction as well as in cattle?

* * *

The need for a reliable standard tuberculin product had been recognized for some years, and in 1934 Long (35) mentioned the essential

characteristics for a standard product: (*1*) a standard tuberculin should have specificity, high potency and constancy; (*2*) it should not give false reactions; (*3*) it should be stable: samples made at different times should be of equal strength; and (*4*) it should not sensitize and thereby cause positive reactions in the absence of infection. Because of the extensive background of usage of Old Tuberculin throughout the world, Long posed the question of whether or not Old Tuberculin itself could be put on a standard basis. That was hardly possible, he said, since

> Old Tuberculin is not a unit substance, but a mixture of extreme complexity. Its potency is due to about one per cent of its total substance. Ninety-nine parts out of a hundred are inert. Whether, in the ninety-nine parts, there are elements present capable of eliciting nonspecific reactions, is debatable, but obviously the absolute specificity of a tuberculin containing protein derivatives from beef- or milk-peptone cannot be guaranteed. Moreover, in all probability, the actual content of active principle is never twice the same.

Green (36) was perhaps a bit more colorful some years later in expressing his opinion about Old Tuberculin:

> It would surely simplify life for manufacturers if O.T. were plainly described as 'any witches' brew derived by evaporation of any unspecified fluid medium in which any unspecified strain of mammalian *M. tuberculosis* had been grown, provided its potency matched that of another witches' brew kept in Copenhagen and called international standard, or any allegedly equivalent sub-standard thereof, when tested on an unspecified number of guinea-pigs without worrying too much about statistical analysis of results.'

For some years, Seibert of the Henry Phipps Institute, Philadelphia, had been concerned with the preparation of a more purified tuberculin product; and in 1932 she and Munday (37) reported the preparation of a precipitated purified tuberculin protein referred to as TPT (trichloracetic acid precipitated tuberculin). This product had a lower molecular weight than the MA-100 protein and, although it was less antigenic than MA-100, it still retained some antigenicity. In tests on guinea pigs, TPT appeared to be potent and fairly specific in low doses, but with doses 1,000 times the usual dose of human TPT, large reactions invariably occurred with both the avian and timothy grass TPT products (26).

Trichloracetic acid precipitated tuberculin was also found to be potent and apparently specific in low doses for tuberculous infection in human beings. Lichtenstein (38), for example, in testing 944 tuberculous adults with serial tenfold dilutions of human TPT, found reactions to the 1:1,000 dilution in 98 per cent, or all but 22, of the patients. And, of the 22 who failed to react to that dose, 13 died within six months, 2 were senile, 5 had far advanced disease, and 2 moderately advanced. Barnwell and Pollard (39) reported similar results from comparative tests with OT and TPT in more than 1,000 persons—college women, high school students, and student nurses. They found that every student with active disease reacted to a weak dose, and concluded: "Thus it has been easily and adequately demonstrated that every proved case of clinical tuberculosis reacts to some dose of T. P. T., but the reverse, that every person so reacting has been infected, is almost impossible of unequivocal proof."

Trichloracetic acid precipitated tuberculin was short-lived. Seibert's continued efforts to obtain a more pure product resulted in 1934 in purified protein derivative (40). Purified protein derivative, called PPD (it had earlier been referred to as SOTT), had a tuberculin protein molecule smaller than that of TPT, and a corresponding reduction of antigenicity. In describing the new product, Long, Seibert, and Aronson (41) made the point that the three strains used in the preparation of PPD were the same three strains which the Bureau of Animal Industry used for the preparation of mammalian tuberculin for veterinary practice. Those strains, as well as Dorset's synthetic medium used by the Bureau of Animal Industry, were chosen for the preparation of PPD

> . . . in order to bring into closer alignment the extensive human and cattle-testing programmes. . . . In this connection it must not be forgotten, however, that there appears to be one kind of infection in cattle, not identical with tuberculosis, leading to skin sensitiveness to tuberculin. This is infection, usually of the skin of legs, with another acid-fast micro-organism. This whole subject is being actively investigated at the present time. Whether anything analogous exists in man is not known, and is also the subject of present research.

In determining the optimal dosage of PPD, Long, Aronson, and Seibert (42) found that 0.00005 mg. and 0.005 mg. were closely comparable with the two commonly used doses of OT, the 1:10,000 and 1:100 dilutions. However, as a dose of 0.00002 mg. appeared to detect a majority of those who reacted to 0.00005 mg., and 250 times that amount did not cause unduly severe reactions in those who failed to react to the first dose, the decision was made to adopt 0.00002 mg. and 0.005 mg. of PPD as the two doses recommended for routine use. These two doses came to be known in the United States as the "first" and "second" doses.

A system of grading reactions to the intracutaneous test with PPD was recommended by Aronson (43) in 1934 and was widely adopted. The reaction was to be read at forty-eight hours after injection and graded as follows:

1+: some redness and definite edema of more than 5 mm. and not exceeding 10 mm.

2+: area of redness and edema measuring from 10 to 20 mm. in diameter

3+: marked redness and edema exceeding 20 mm. in diameter

4+: marked redness, edema, and an area of necrosis

Doubtful: slight redness and trace of edema measuring 5 mm. or less

Negative: no edema at injection site, even if slight redness is present.

Clearly, the development of PPD, a purified, potent, stable tuberculin without sensitizing properties, was an important landmark in the history of tuberculin. The enormous amount of testing done with PPD has amply demonstrated its practical usefulness, and in 1941 Seibert and Glenn (44) made a single large lot for deposit as an official standard tuberculin. The method for preparing this standard lot involved certain modifications of the original method, including the use of Long's synthetic medium instead of Dorset's medium, a single strain of human tubercle bacillus from the Bureau of Animal Industry rather than three strains, and final precipitation with ammonium sulfate instead of trichloracetic acid. This lot, known as PPD-S, was established as the International Standard for Purified Protein Derivative of Mammalian Tuberculin by the WHO Expert Committee on Biological Standardization (45).

The question of the specificity of reactions to tuberculin, and particularly to the strong doses of tuberculin, was still dogging the footsteps of some workers, even after the introduction of PPD. In the minds of many persons, "purified" tuberculin was synonymous with "specific" tuberculin. The studies of McCarter, Getz, and Stiehm (46) threw strong doubts on that as-

TABLE 3

REACTIONS OF MALE UNIVERSITY STUDENTS IN WISCONSIN TO HUMAN AND AVIAN PPDs, AND ROENTGENOGRAPHIC FINDINGS OF THE REACTORS

(Adapted from McCarter et al., Am. J. M. Sc., 1938, 195, 479)

Classification		Number of Roentgenograms	Interpretation of Roentgenograms (Percentage)			
			Normal	Calcification	Infiltration	Active Tuberculosis
	Percentage					
739 freshmen						
Human + and avian +	23.8	172	68.0	23.8	6.4	1.8
Human + and avian −	0.4	3	—	0.0	—	0.0
Human − and avian +	22.3	161	90.7	8.1	1.2	0.0
Human − and avian −	53.5	0				
	Percentage					
125 agricultural students						
Human + and avian +	21.6	27	74.1	25.9	0.0	0.0
Human + and avian −	0.8	1	—	0.0	0.0	0.0
Human − and avian +	33.6	0				
Human − and avian −	44.0	0				

sumption. More than 1,000 university and agricultural students in Wisconsin were tested with the standard first and second doses (0.00002 mg. and 0.005 mg.) of PPD tuberculins prepared by Seibert from human, avian, and bovine tubercle bacilli; tests were also given with a smegma bacillus PPD and with the two "standard" doses of OT (0.01 and 1.0 mg.). Roentgenograms were obtained of students with positive reactions to one or more of the tests. The results were provocative. Almost all of the students who reacted to human PPD also reacted to avian PPD, as shown in table 3. Although the data were not shown, the writers stated that all strong reactors to human PPD (that is, those who reacted to the first dose or who had either 3-plus or 4-plus reactions to the large dose) also reacted to avian PPD, but with a smaller reaction; whereas weak reactors to human PPD (1-plus or 2-plus to only the large dose) had reactions of about the same size to avian PPD. Moreover, as shown in the table, an equal proportion of the university students and an even greater proportion of the agricultural students reacted to the avian but not to the human PPD; and, surprisingly, among them some were found to have pulmonary calcifications (which, at that time, were attributed to tuberculous infection). While these results posed numerous problems, the writers concluded that:

> The qualitative specificity of the tuberculin reaction must be questioned; we cannot prove with our present knowledge that all reactions to human P.P.D. are due to sensitization by the human or bovine tubercle bacillus, and we know that some individuals with healed lesions of tuberculosis do not react to human P.P.D. We cannot prove that individuals with weak reactions to human P.P.D., *i.e.*, 1+ or 2+ to the strong dose of 0.005 mg., have been sensitized by the human or bovine tubercle bacillus, because such individuals react in about the same degree to the P.P.D.'s of other acid-fast bacteria such as the avian tubercle bacillus. . . .

Thus, by the mid 1930's, the evidence was beginning to grow that human beings, like cattle, might be subject to nonspecific (nontuberculous) sources of sensitization to human tuberculin. The traditional belief of the medical profession in the specificity of all reactions to tuberculin was being seriously questioned for the first time. While it still appeared to be true that persons who had been infected by tubercle bacilli reacted to tuberculin, there was now good reason to doubt that all persons who reacted to tuberculin had had tuberculous infections. The medical profession was beginning to learn what some of the veterinarians had known for at least ten years.

CHAPTER V

THE CASE OF PULMONARY CALCIFICATIONS IN TUBERCULIN NONREACTORS

Admittedly, studies conducted during the late 1930's on the specificity of the tuberculin reaction were not simplified by what later proved to be an extraneous finding, pulmonary calcifications, interpreted as healed tuberculous lesions, in some nonreactors to tuberculin. The presence of calcifications in tuberculin nonreactors had been noted by numerous investigators, prominent among whom were McPhedran and Opie (47); and by 1939 the problem had become acute—and highly controversial as well—with grave doubts of the value of the tuberculin test for measuring the prevalence of tuberculous infection at different times and in different places.

Gass and his colleagues (48) reported from a careful study of more than 1,000 white and Negro school children in Williamson County, Tennessee, that pulmonary calcifications were almost as frequent in tuberculin-negative as in tuberculin-positive subjects. Roentgenograms of all of the children had been read independently by two persons and without knowledge of the results of the tuberculin tests. The findings are summarized in tables 4 and 5. The frequency of calcifications was practically the same in both the positive and negative subjects; and it was only a little higher among those who reacted to the first dose than among those who reacted only to the strong dose of Old Tuberculin. Similar results were reported from Xenia, Ohio, where Nelson, Mitchell, and Brown (49) found approximately the same frequency of pulmonary calcifications, 43 per cent, in both the tuberculin-negative and tuberculin-positive subjects; and from Giles County Tennessee, where Dearing (50) found calcifications in 34 per cent of the negative and in 35 per cent of the positive subjects.

The explanation commonly offered to account for these disturbing results was that tuberculin sensitivity disappeared with complete healing of the tuberculous focus. This view was supported by the studies of Feldman and Baggenstoss (51), which showed that primary lesions, when encapsulated and sclerotic, or caseous or caseocalcareous, seldom contain viable tubercle bacilli. Dahlstrom's studies (52) of the variability of tuberculin allergy were also interpreted as supporting this explanation. From the dispensary records at the Henry Phipps Institute for nearly 3,000 persons, members of tuberculous families who had been observed for at least five years, Dahlstrom found, as shown in table 6, that among 2,490 persons who were considered positive at one time, 276 (11.1 per cent) became negative, either transitorily or for the duration of the study period. Most of those who apparently lost their allergy were either second-dose reactors or weak reactors to the first dose. Conversely, the stronger the initial degree of sensitivity, the less likely was a loss of sensitivity. Only 18 (about 1 per cent) of 1,810 who reacted with at least 10 mm. (4-, 3-, or 2-plus) to the first-dose test appeared to lose their allergy as compared with a loss in nearly 38 per cent (257 of 680) of those who reacted with less than 10 mm. (1-plus) to the first-dose test or only to the second-dose test.

TABLE 4

PERCENTAGE FREQUENCIES OF ROENTGENOGRAPHIC FINDINGS FOR 1,300 WHITE AND NEGRO CHILDREN IN TENNESSEE, BY RESULTS OF TUBERCULIN TEST

(Adapted from Gass et al., Am. Rev. Tuberc., 1938, 38, 441)

Roentgenographic Findings	White		Negro	
	Tuberculin-positive	Tuberculin-negative	Tuberculin-positive	Tuberculin-negative
Negative	33.2	38.2	38.4	51.1
Doubtful	15.7	13.7	12.6	14.3
Definite calcified lesions	51.1	48.1	49.0	34.6
Limited	35.0	33.2	28.0	20.3
Extensive	16.1	14.9	21.0	14.3
Total	100.0	100.0	100.0	100.0

TABLE 5

PERCENTAGE FREQUENCIES OF ROENTGENOGRAPHIC FINDINGS FOR 452 TUBERCULIN-POSITIVE WHITE CHILDREN IN TENNESSEE, BY DEGREE OF TUBERCULIN SENSITIVITY

(Adapted from Gass et al., Am. Rev. Tuberc., 1938, 38, 441)

Roentgenographic Findings	Positive to 0.01 Mg. of OT		Two-plus or More Reaction to 1.0 Mg. of OT		One-plus Reaction to 1.0 Mg. of OT	
	Number	Percentage	Number	Percentage	Number	Percentage
Negative	24	28.6	21	28.0	105	35.8
Doubtful	9	10.7	13	17.3	49	16.7
Definite calcified lesions	51	60.7	41	54.6	139	47.4
Limited	32	38.1	28	37.3	98	33.4
Extensive	19	22.6	13	17.3	41	14.0
Total	84	100.0	75	99.9	293	99.9

Dahlstrom's findings have been repeated by other workers, who found that apparent loss of sensitivity to tuberculin rarely, if ever, occurs in persons who are highly sensitive to tuberculin, i.e., strong first-dose reactors. Most of the so-called "reversions" are observed in persons with a low degree of sensitivity; that is, those with small reactions to the first dose or who react only to the second-dose test.

The discovery of a very prevalent nontuberculous cause of pulmonary calcifications in some parts of the United States is a familiar story. At a conference held in Hagerstown, Maryland, in 1938, and attended by research workers from the Henry Phipps Institute, the National Tuberculosis Association, the U. S. Public Health Service, and others, it was agreed that studies were needed on the relation between pulmonary calcification and tuberculin sensitivity and that these studies should be carried out in several different places. The major question upon which opinion was divided concerned the specificity of the tuberculin reaction. While some concurred with the commonly accepted belief that waning of allergy was directly related to the calcification process and represented the healing of a primary tuberculous lesion, a few were struck by the discrepancy between the high frequency of pulmonary calcifications in tuberculin-negative persons in some geographic areas and the low frequency in others. How could that be, they reasoned, if every calcified lesion represents a healed primary tuberculous focus? They believed it more likely that some cause other than tuberculosis was producing calcifications in a high proportion of the population in some areas and in a negligible proportion in others. Although Long (53) believed that ". . . most of us have tended to overlook the fact that with the arrest and healing of tuberculous lesions allergy wanes and finally may disappear," he also recognized the possibility that

TABLE 6

INSTABILITY OF THE TUBERCULIN REACTION IN RELATION TO PREVIOUS STRENGTH OF REACTION

(Reproduced with permission from Dahlstrom, Am. Rev. Tuberc., 1940, 42, 471)

Dose of Tuberculin	Number	Strongest Reaction	Became Negative	
			Number	Per Cent
First:				
OT, 0.01 mg.; or PPD, 0.00002 mg.	28	4+	0*	0.0
	1,090	3+	4	0.4
	692	2+	14	2.0
	320	1+	40	12.5
Second:				
OT, 0.1 mg.; OT, 1.0 mg.; or PPD, 0.005 mg.	None	4+		
	66	3+	18	27.3
	109	2+	68	62.4
	185	1+	131	70.8
Total of once-positive reactors	2,490		276	11.1
Remained negative throughout	226			8.0
Examined once, found negative	112			4.0
Total	2,828			

* The original table showed one person here who was later found to have been included by mistake (Dahlstrom, personal communication).

pulmonary calcifications might result from causes other than tuberculosis (54).

On the basis of the latter possibility, a search was made for other causes of pulmonary calcifications. Earlier, in 1936, Carter (55) had reported calcified pulmonary lesions associated with the fungal infection coccidioidomycosis, which is highly prevalent in the southwestern part of the United States; and in 1942, Aronson and his associates (56) reported pulmonary calcifications which they believed could be attributed to coccidioidal infection among coccidioidin-positive, tuberculin-negative Indians living in the Southwest. Three years later, Palmer's nationwide studies of tuberculosis in student nurses (57) revealed that a very high proportion of tuberculin nonreactors with pulmonary calcifications occurred in the east-central states, centering around the Kansas City area. Tests with coccidioidin ruled out Coccidioides as a possible cause; but tests with histoplasmin showed that the wide geographic variations in prevalence of pulmonary calcifications corresponded with wide variations in prevalence of histoplasmin sensitivity and, in addition, that there was a high degree of correlation between histoplasmin sensitivity and pulmonary calcifications in tuberculin nonreactors. Christie and Peterson (58) reported a similar correlation between pulmonary calcification and histoplasmin sensitivity among tuberculin-negative children in Nashville, Tennessee; and subsequently, Furcolow (59) was able to demonstrate the gradual calcification of pulmonary infiltrates in histoplasmin-positive, tuberculin-negative children from whom the fungus *H. capsulatum* was cultured.

Today, it is well known that histoplasmosis, which is generally a benign self-limiting infection, and also coccidioidomycosis account for a large proportion of the pulmonary calcifications found in persons living in the regions where these fungal infections are highly prevalent.

CHAPTER VI

RECOGNITION OF "NO-LESION REACTORS" IN HUMAN BEINGS

For many workers, faith in the specificity of the tuberculin test was restored with the solution of the pulmonary calcification problem. But evidence obtained during the course of the studies on calcification again pointed to the nonspecific nature of reactions to strong doses of tuberculin. Furcolow and his associates (60), in 1941, published results of a quantitative titration of tuberculin sensitivity in relation to tuberculous infection in a study of various population groups in Ohio and Minnesota. They found a close association between the degree of tuberculin sensitivity, as indicated by tests with a twelve-dose series of tenfold dilutions of PPD tuberculin, and the presence of active tuberculous disease, or known contact with tuberculosis. Furthermore, they found that almost everyone would react to tuberculin if sufficiently strong doses were given —even infants less than six months of age with little likelihood of having had contact with tuberculosis. Where, then, was the limit of dosage that would detect only the tuberculosis-infected?

Figure 1, adapted from Furcolow and associates (60), appears to set the limit at about 0.0001 mg. of PPD, a dose five times larger than the customary first-dose test of 0.00002 mg. and fifty times smaller than the second-dose test of 0.005 mg. The range of tenfold dilutions from 0.000001 mg. to 1.0 mg. of PPD is plotted along the bottom of the figure; the cumulative percentage of reactors (5 or more mm. of induration) up the side. Results for different groups are represented by separate lines in the graph and are also given in table 7. Line *A*, for example, is for a group of 60 children, all less than fifteen years of age, who were hospitalized for active tuberculosis. Many reacted to doses smaller than 0.000001 mg. and, with added reactors as the dose was increased, a cumulative total of 93 per cent reacted to 0.00001 mg., and 100 per cent to 0.0001 mg. Similarly, line *B* shows that among 468 white and Negro tuberculous patients of both sexes, aged fifteen to seventy years, 99.6 per cent reacted to a dose of 0.0001 mg. or less.

In contrast, among 32 infants less than six months old with no known contact with tuberculosis (line *H*) none reacted to 0.0001 mg., 0.001 mg., or even to 0.01 mg.; but some reacted to 0.1 mg. and some to 1.0 mg., giving a cumulative total of 72 per cent reactors to the strongest dose. Lines *G*, *F*, and *E* are for children of increasing age and show that the proportion of reactors to 0.0001 mg. or less gradually increased as the likelihood of contact with tuberculosis increased as the children grew older; yet, close to 95 per cent reacted to the strongest dose, regardless of age.

These findings raised several significant points about the interpretation of reactions to tuberculin. First, nearly everyone in the total study population could be called "positive" to tuberculin if strong enough doses were used; but, in general, persons who reacted only to the strongest doses did not have tuberculous disease or any known contact with tuberculosis. If these reactions to strong doses were specific, the writers reasoned, ". . . it would be necessary to assume that almost all persons tested, even very young infants, had been infected with the tubercle bacillus. Such an assumption seems unwarranted." Moreover, the strong-dose reactions were much less stable over a period of time than reactions to the weaker doses, a finding which supported Dahlstrom's observation that "reversion" of sensitivity was relatively common in strong-dose reactors but rare in weak-dose reactors. Finally, in agreement with earlier studies by various workers, Furcolow and his associates found that most persons with tuberculosis, and a large proportion of persons who had had contact with tuberculosis, reacted to a relatively weak dose of tuberculin. The authors concluded that the reactions which are brought out only by very strong doses of tuberculin are probably nonspecific ". . . or, in other words, that they are not due to infection with the tubercle bacillus." And an increasing number of nonspecific reactions was obtained as the strength of the dose of tuberculin was increased.

Since other workers had also found that the 0.0001-mg. dose of PPD elicited a positive reaction in almost all persons with tuberculous disease, as well as in most of those who had presumably had tuberculous infections, and the reaction to that dose was negative in most persons who presumably had not been infected,

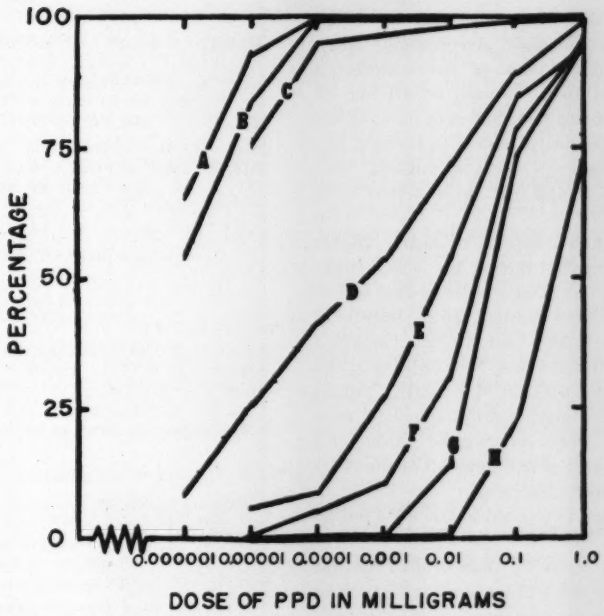

FIG. 1. Cumulative percentages of reactors to serial doses of PPD for various population groups in Ohio and Minnesota. (*Data plotted from table 7*)

TABLE 7

CUMULATIVE PERCENTAGES OF REACTORS TO SERIAL DOSES OF PPD FOR DIFFERENT POPULATION GROUPS IN OHIO AND MINNESOTA

(*Adapted from Furcolow et al., Pub. Health Rep., 1941, 56, 1082*)

Population Group	Number of Persons Tested	Cumulative Percentages of Reactors to Dose (in Mg.) of:							Presence of Active Tuberculosis or Contact with Tuberculosis
		0.000001	0.00001	0.0001	0.001	0.01	0.1	1.0	
A. Tuberculous patients Age 0–14 years	60	65.0	93.3	100.0	—	—	—	—	All had active tuberculosis
B. Tuberculous patients Age 15–70 years	468	53.4	83.8	99.6	99.6	100.0	—	—	All had active tuberculosis
C. Mental patients Age 15–60 years	499	—	76.2	95.6	97.2	98.2	98.8	99.6	10% had tuberculosis; the others lived in close contact
D. Antituberculosis camp Age 0–14 years	101	8.9	25.7	41.6	53.5	72.3	90.1	99.0	60% were reported to have had history of contact
E. Orphanage Age 6–19 years	248	—	6.5	8.5	26.6	53.6	84.7	93.5	No history of contact
F. Foundling home Age 4–6 years	19	—	0	5.3	10.5	31.6	78.9	94.7	No known contact
G. Foundling home Age 6 months to 3 years	65	—	0	0	0	13.8	73.8	95.4	No known contact
H. Foundling home Age less than 6 months	32	—	0	0	0	0	21.9	71.9	No known contact

the 0.0001-mg. dose was accepted as being a reasonably specific dose. Accordingly, when Palmer began his studies of tuberculosis in student nurses in 1943, he used 0.0001 mg. of PPD-S for the routine test; but in order to study further the significance of reactions brought out only by a much stronger dose, all students who did not react to 0.0001 mg. were tested with 0.005 mg.

The study included a total of nearly 26,000 white students enrolled during the years 1943–1949 in 76 nurses' training schools located in ten different metropolitan centers, from Baltimore and Philadelphia in the East, to San Francisco and Los Angeles in the West, New Orleans in the South, and Minneapolis in the North. Tuberculin tests and roentgenograms of the chest were given routinely every six months throughout the three-year period of training. All of the chest roentgenograms were reviewed by one roentgenologist who was not permitted to know the results of the skin tests.

The 0.0001-mg. or 5-TU dose of PPD-S[7] was shown to be highly efficient for identifying persons who had pulmonary infiltrates or calcifications and who did not react to histoplasmin (61, 62). Shadows interpreted as calcifications or parenchymal infiltrates were, as shown in table 8, many times more frequent in reactors to 5 TU than in either reactors or nonreactors to the strong (250 TU) dose. In fact, the frequencies of lesions seemed to be unrelated to whether or not the person reacted to the strong-dose test.

Similar, almost identical, results had been reported earlier by Scheel (63) in Norway. Among nearly 1,700 university students in Oslo, calcified foci were found in about 15 per cent of those with positive reactions (3 or more mm.) to the Pirquet test; in contrast, among those with no reactions (0 mm.) to both the Pirquet test and the Mantoux test with 1 mg. of OT, only 0.7 per cent had calcifications; and among the Pirquet negative–Mantoux positive reactors, the frequency was 1 per cent. Thus, Scheel had also found a pronounced, direct relation between pulmonary calcification and a high degree of sensitivity to tuberculin and virtually no relation between calcification and the low-grade sensitivity brought out only by a strong dose (1 mg.) of OT.

[7] For simplification, tuberculin dosage for PPD will hereafter be expressed in terms of the accepted international tuberculin units. One tuberculin unit (1 TU) is defined as 0.00002 mg. of PPD-S. The 0.0001-mg. dose of PPD-S is, therefore, 5 TU; the 0.002-mg. dose is 100 TU; and the 0.005-mg. dose is 250 TU.

TABLE 8

PERCENTAGES OF 13,000 STUDENT NURSES* WITH PULMONARY INFILTRATES OR CALCIFICATIONS, ACCORDING TO SENSITIVITY TO 5 TU OR 250 TU OF PPD-S

(Adapted from Edwards et al., Pub. Health Rep. 1948, 63, 1569; and Goddard et al., Pub. Health Rep., 1949, 64, 820)

Classification of Reactors	Percentage with:	
	Infiltrates	Calcifications
Positive to 5 TU	3.5	10.7
Positive to 250 TU (among those negative to 5 TU)	0.3	0.7
Negative to 250 TU	0.4	0.7

* Excluding all reactors to histoplasmin.

TABLE 9

PERCENTAGES OF REACTORS TO 5 TU OR 250 TU IN 16,000 STUDENT NURSES CLASSIFIED BY HISTORY OF CONTACT WITH TUBERCULOSIS

(Adopted from Palmer, Am. Rev. Tuberc., 1953, 68, 678)

Classification	No Contact	Intermediate Contact	Close Contact	Total
Positive to 5 TU	11.4	20.4	41.4	13.1
Positive to 250 TU (among those negative to 5 TU)	33.5	33.8	33.6	33.6

The relation between history of contact with tuberculosis and sensitivity to tuberculin was also studied by Palmer (64) in his student nurse group. At the time of her admission to training school, each student had been asked for detailed information about previous known contact with a case or cases of tuberculosis in her home or among relatives, friends, or classmates. According to their histories of contact, the girls were grouped into three categories representing, as well as could be judged, decreasing likelihood of having been infected: those with history of close contact in the home; those with contact outside the home or contact with an arrested

TABLE 10

PERCENTAGES OF REACTORS TO 5 TU OR TO 250 TU IN 16,000 STUDENT NURSES CLASSIFIED BY HISTORY OF CONTACT WITH TUBERCULOSIS AND PLACE OF RESIDENCE

(Adopted from Palmer, Am. Rev. Tuberc., 1953, 68, 678)

Place of Residence	No Contact	Intermediate Contact	Close Contact	All Contact Groups
Positive to 5 TU				
Northern and western states	10.1	19.1	41.0	11.7
Southeastern states	12.2	19.3	44.0	13.3
All states	10.3	19.1	41.2	11.9
Positive to 250 TU (among those negative to 5 TU)				
Northern and western states	28.9	29.3	27.0	28.9
Southeastern states	66.8	68.8	70.0	67.0
All states	32.5	33.2	29.3	32.5

case; and those with no known contact. The grouping was of course made without knowledge of the tuberculin test results.

The percentage of reactors to 5 TU at the time of admission increased as the likelihood of previous infection increased. As shown in table 9, 11.4 per cent in the no-contact group were reactors, 20.4 per cent in the intermediate-contact group, and 41.4 per cent in the close-contact group. In contrast, among the girls in the three groups *who did not react to the 5-TU test*, the frequency of reactors to 250 TU was almost exactly the same (nearly 34 per cent) regardless of contact. Thus, contact with tuberculosis had a pronounced effect on sensitivity to 5 TU but had no effect on the frequency of reactors to 250 TU.

The most potent factor affecting frequency of reactors to 250 TU was geography—where a person lived. Of the total study population, 70 per cent could be classified as permanent residents of some one state; and for these students results were presented for those from the northern and western states as one group, and for those from the southeastern states as another. Within each broad geographic group, as shown in table 10, the results were further subdivided by history of contact with tuberculosis. Place of residence did not affect the total percentage of reactors to the 5-TU test. With the increasing likelihood of infection, the frequency of reactors in both areas increased from a little more than 10 per cent in the no-contact groups to 19 per cent in the intermediate and 41 per cent in the close-contact groups. The picture was very different for reactors to the 250-TU test. The percentage of reactors to the strong dose (among those who did not react to 5 TU) was more than twice as high in residents from the southeastern states as in those from the North and West, and was entirely unrelated to history of contact with tuberculosis. Thus, the percentage of reactors to 250 TU showed wide geographic variations while the percentage of reactors to 5 TU was much less variable. This was regarded as strong evidence that sensitivity to a strong dose of tuberculin did not have the same cause as sensitivity to 5 TU.

Another striking illustration of the geographic differences in the frequency of reactors to weak and strong doses of tuberculin is given in figure 2 (adapted from Palmer (65)). The figure shows the results of tests with 5 TU and 100 TU of PPD-S for about 7,500 white children in Muscogee County, Georgia, and nearly 1,000 white children in Lapeer, Michigan. The age of the children is

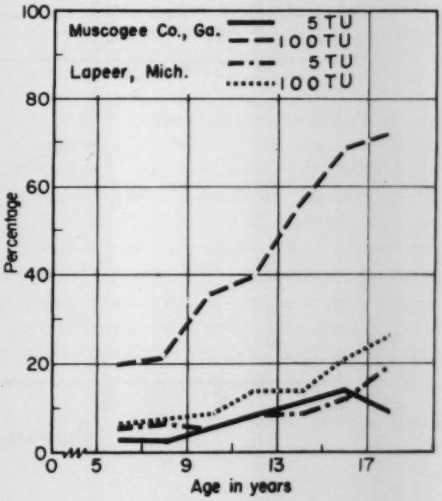

FIG. 2. Percentages of reactors to 5 or 100 TU of PPD, by age, for 8,500 school-age children in Georgia and Michigan. *(Adapted from Palmer, Tr. Nat. Tuberc. A., 1952, p. 705)*

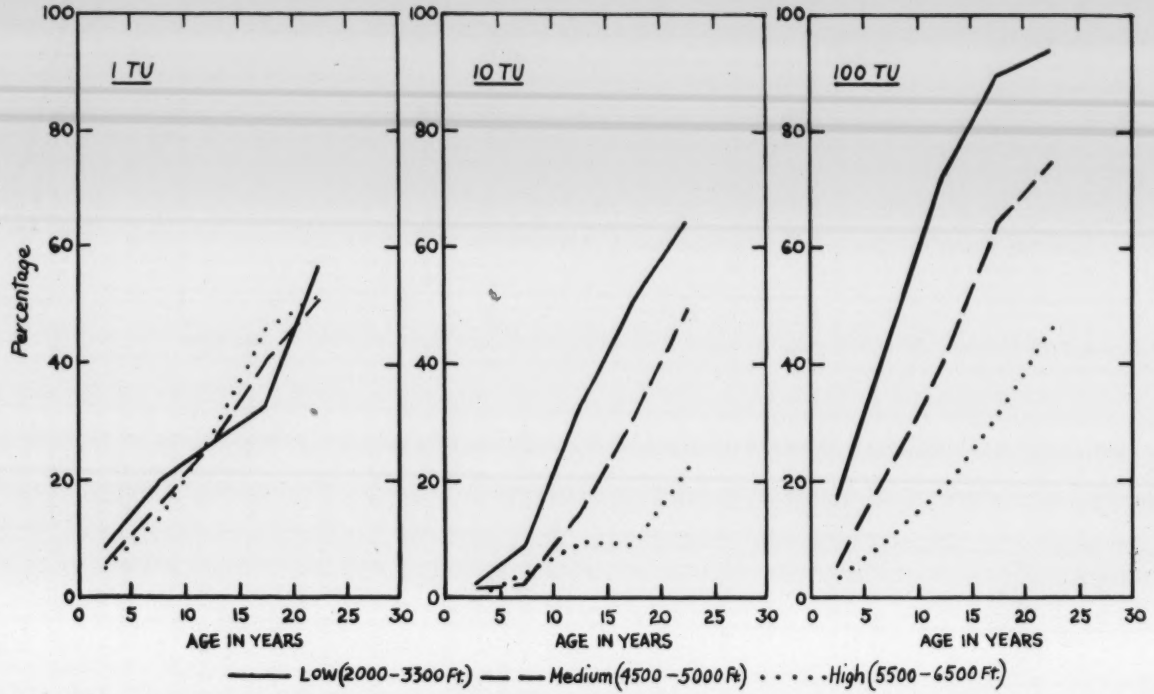

FIG. 3. Percentages of reactors to 1, 10, or 100 TU of PPD (RT), by age and altitude of residence for 4,000 persons living in Darjeeling. (*Reproduced from Bates et al., Pub. Health Rep., 1951, 66, 1427.*)

plotted along the bottom of the graph, the percentage of reactors along the side. As shown by the two lower curves, the percentage of reactors to 5 TU was nearly the same in both areas, starting around 5 per cent at six years of age and rising to almost 15 per cent at sixteen years. When reactors to either 5 TU or 100 TU are combined for each area, illustrated by the two upper curves, there is an enormous increase in the percentage of reactors in Georgia compared with only a small increase in Michigan. The difference is due to the high proportion of children living in Georgia who have sensitivity which is brought out only by a strong dose of tuberculin, whereas that kind of sensitivity is apparently uncommon among children in Michigan.

The prevalence of strong-dose sensitivity has also been shown to vary with altitude above sea level in some localities, as revealed in a study by Bates and co-workers (66) of approximately 4,000 children and young adults living on tea plantations in a mountainous area near Darjeeling in northern India. All but one of the nine plantations were within three air miles of one another on the mountain slopes, yet their altitudes ranged from 2,000 to 6,500 feet above sea level. Tests were given first with 1 TU of PPD,[8] and those who did not react were given 10 TU; the nonreactors to that dose were given 100 TU. The results are shown in figure 3 and table 11. The frequency of reactors to the 1-TU dose was similar at all altitudes of residence. But, with the 10-TU test, the curves began to spread apart, and with the strong dose they became widely separated. In the oldest age group (twenty to twenty-four years), for example, 94 per cent of the low-altitude residents who did not react to either 1 or 10 TU reacted to 100 TU as compared with 46 per cent for the corresponding group among high-altitude residents.

TABLE 11

PERCENTAGES OF REACTORS TO 1 TU, 10 TU, OR 100 TU OF PPD (RT), BY AGE AND ALTITUDE OF RESIDENCE, IN 4,000 YOUNG PERSONS LIVING IN DARJEELING

(*Adopted from Bates et al., Pub. Health Rep., 1951, 66, 1427*)

Age	1 TU			10 TU*			100 TU†		
	Altitude of residence								
	Low	Medium	High	Low	Medium	High	Low	Medium	High
years									
0–4	8.1	5.3	4.6	2.2	1.2	0.0	16.8	5.1	3.3
5–9	19.8	15.5	13.3	9.1	2.7	4.4	44.7	22.1	10.7
10–14	26.7	26.7	28.1	33.6	15.2	9.7	72.5	40.8	19.7
15–19	32.9	40.4	45.4	50.9	30.6	9.2	89.8	64.5	31.6
20–24	57.1	50.9	51.0	64.6	49.4	22.2	94.1	75.0	46.4

* Given to those who were negative to 1 TU.
† Given to those who were negative to 10 TU.

Palmer (64) believed that the results of all of these studies were consistent with the hypothesis that

> . . . tuberculin sensitivity in human beings can no longer be regarded as being derived from a single source. Most reactions elicited by a low-dose intradermal test (approximately 0.0001 mg. of PPD) undoubtedly indicate specific infection with virulent tubercle bacilli, spread by personal contact. Low degrees of sensitivity brought out only by large doses (1 mg. of OT or 0.005 mg. of PPD, for example) apparently represent infection by a different organism with a different mode of transmission. The latter (unidentified) organism must be antigenically related to the tubercle bacillus, highly prevalent in certain geographic areas, and apparently nonpathogenic for human beings.

With recognition that the small 5-TU dose of tuberculin will miss only an occasional person infected with tubercle bacilli, large-scale public health programs gradually abandoned the second or strong-dose test. And the way was paved for further advances in the understanding of tuberculin sensitivity with the accumulating evidence that most of the reactions brought out only with the strong doses (100 TU and 250 TU) probably are not caused by infection with virulent tubercle bacilli.

[8] Lot RT XIX-XX-XXI from the State Serum Institute, Copenhagen.

CHAPTER VII

QUANTITATIVE ASPECTS OF TUBERCULIN SENSITIVITY

Anyone who has given more than a few tuberculin tests knows well that not all persons react in the same degree to a particular dose. Some have unusually large reactions, some have moderate reactions, some do not react at all. If the tuberculin reactions of any large group of tuberculous patients are carefully measured, however, the reactions will be found to be distributed over a fairly broad range in size, but the bulk of them will be grouped closely around the mean. This is illustrated in table 12, which gives a tabulation of the sizes of induration of reactions to an intracutaneous test with 5 TU of PPD-S for a group of patients hospitalized for tuberculosis at the Battey State Hospital, Georgia (67).

Most of the reactions of these 1,592 patients are concentrated in the middle of the size range, the greatest numbers falling between 15 and 22 mm. Below 15 mm., the numbers taper off rapidly, with very few reactions at the extreme lower end of the scale; and above 22 mm., the numbers again taper off toward the upper end of the scale. The mean reaction size for the entire group is a fraction over 18 mm., with more than 70 per cent of all reactions within 5 mm. of the mean. It would not be surprising, therefore, after reading these tuberculin reactions, to have a distinct impression that the "typical" reaction for these patients is between 16 and 20 mm. in diameter.

The same results, expressed in terms of percentages, are presented graphically as a frequency histogram in figure 4. The pattern of the distribution is perhaps seen more clearly here than from the column of figures given in the table. To reduce gross (and meaningless) irregularities resulting from reading bias—the unconscious tendency of a reader to record certain (usually round) numbers more often than other numbers—the percentages are plotted for 2-mm. groupings rather than for each single millimeter. Reading from the histogram, it may be seen that 0.2 per cent of the entire group had reactions of either 0 or 1 mm., and are represented by the first vertical bar. The highest bar indicates that 19.9 per cent of the patients had reactions of 16 or 17 mm. Only 2.3 per cent had reactions of 30 mm. or more. The resulting histogram forms a fairly symmetrical, dome-shaped pattern resembling the well-known "normal" curve.

Results for other groups of patients hospitalized for tuberculosis in various parts of the world, reported by the WHO Tuberculosis Research Office (68), are reproduced in figure 5. Each group of patients (except in the United States) was tested intracutaneously with a dose of 5 TU of the purified protein derivative identified as RT XIX–XX–XXI from the State Serum Institute in Copenhagen; the American patients were tested with 5 TU of PPD-S.[9] Aside from small irregularities, each distribution approximates the normal curve, the mean size of the reactions ranging from 12.8 mm. for patients in south India to 18.8 mm. for patients in the Republic of the Sudan. The difference between the smallest and largest mean size is thus 6 mm., or about a quarter of an inch. As shown in figure 6, the remarkable similarity of the pattern of these distributions suggests that sensitivity to the 5-TU dose of tuberculin is probably a universal characteristic of persons with clinical tuberculous disease, despite such obvious factors as age, race, sex, kind or stage of disease, nutritional status, et cetera, that may otherwise cause the groups of patients to differ considerably from one another.

The same kind of pattern is often found in the distribution of reactions of general population groups, but with the addition of a second concentration of reactions at the far left or zero end of the size scale. A report in 1955 by Groth Petersen and Wilbek (70), from the Danish Tuberculosis Index in Copenhagen, provides a clear example of the bimodal or two-peaked distribution which may be obtained in testing a large general population with a low dose of tuberculin, in this case 10 TU of Danish PPD. In figure 7, reproduced from that report, the results are shown (grouped according to age) for the adult Danish population of four districts of the country included in the nation-wide tuberculosis mass campaign of 1950–1952. For each age group, the distribution is clearly composed

[9] These two PPD products have been shown by Nissen Meyer (69) to be very similar in the low-dosage range of 1 to 10 TU.

TABLE 12

DISTRIBUTION OF SIZES OF REACTIONS TO 5 TU OF PPD-S IN PATIENTS IN A TUBERCULOSIS HOSPITAL IN GEORGIA

(*Adapted from Palmer and Bates, Bull. World Health Organ., 1952, 7, 171*)

Transverse Diameter of Induration	Number of Persons	Percentage	
		For Single Millimeters	For 2 Millimeter Groupings
mm.			
0	3	0.2	
1	—	—	0.2
2	3	0.2	
3	4	0.3	0.5
4	7	0.4	
5	3	0.2	0.6
6	1	0.1	
7	10	0.6	0.7
8	13	0.8	
9	17	1.1	1.9
10	24	1.5	
11	64	4.0	5.5
12	56	3.5	
13	50	3.1	6.6
14	61	3.8	
15	111	7.0	10.8
16	168	10.7	
17	147	9.2	19.9
18	90	5.7	
19	104	6.5	12.2
20	101	6.3	
21	146	9.2	15.5
22	128	8.0	
23	66	4.1	12.1
24	34	2.1	
25	57	3.6	5.7
26	29	1.8	
27	32	2.0	3.8
28	15	0.9	
29	13	0.8	1.7
30	6	0.4	
31	9	0.6	1.0
32	10	0.6	
33	3	0.2	0.8
34	2	0.1	
35	—	—	0.1
36	—	—	
37	1	0.1	0.1
38	—	—	
39	1	0.1	0.1
40 and over	3	0.2	0.2
Total	1,592	100.0	100.0

Mean size of reactions—18.3 mm.
Standard deviation—5.3 mm.

of two segments. The segment toward the right is composed of reactions ranging from about 6 mm. to more than 26 mm., and with a peak at 14 to 15 mm. This group closely resembles the reactions of tuberculous patients and is interpreted as representing the tuberculosis-infected segment of the population. The other group, which consists of reactions concentrated at 0 or 1 mm. and includes reactions up to 5 mm. in size, is considered the uninfected segment of the population.

In support of that interpretation, the writers show the distribution of tuberculin reactions for 344 new cases of active tuberculosis found in the first counties covered by the mass campaign. These new cases were persons whose photofluorograms were thought to show suspicious shadows[10] and who were subsequently diagnosed as having active disease when further examination revealed cultures positive for tubercle bacilli. The distribution of reactions for these 344 previously unknown cases, shown in figure 8, corresponds closely to the form and position on the size scale of the right-hand segment of the distribution for the general population of 50,000 persons. There can be little doubt that most of the persons who have had tuberculous infections, including those with active tuberculous disease, will, in fact, be found in the group who react with 6 mm. or more to a low dose test.

Finally, analysis of the Danish mass campaign material showed that the frequency of pulmonary calcifications (except for the age groups over forty-five years) was very low in persons with reactions of less than 6 mm.; it rose sharply among persons with reactions of 8 to 9 mm.; then increased progressively and leveled off at maximal frequency in persons with reactions of 18 mm. and larger. As Histoplasma infection has never been found in Denmark and several testing surveys have failed to reveal any evidence of histoplasmin sensitivity in the population, pulmonary calcification is considered a reliable sign of tuberculous infection in Denmark. The writers thus had good reason to believe that:

> The significant abrupt rise in the calcification frequency at the reaction size of 8–9 mm is strong evidence that the natural separation

[10] During the Danish campaign, photofluorograms of all adults were obtained simultaneously with the tuberculin tests.

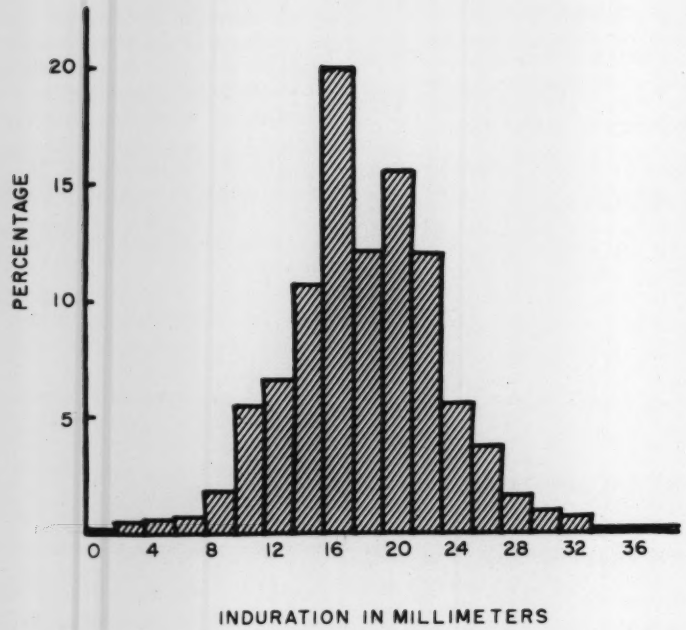

FIG. 4. Distribution of sizes of reactions to 5 TU of PPD-S for 1,592 patients in a tuberculosis hospital in Georgia. (*Data plotted from table 12*)

of two groups according to size of reaction to the 10 TU test corresponds very well to a separation into infected and uninfected.

The distribution of tuberculin reactions, however, does not always form a clear bimodal pattern. The material in figure 9, which was reported by the WHO Tuberculosis Research Office (68, 71), shows the reactions to 5 TU and 100 TU for groups of more than 4,000 school children in Denmark, nearly 2,000 school children in the Philippines, and 900 school children in the State of Travancore-Cochin on the southwestern coast of India. The 100-TU test was given only to those who reacted with less than 5 mm. of induration to the 5-TU test.

In comparing the three histograms of reactions to 5 TU, it is immediately evident that the situations in Travancore-Cochin and Denmark are widely different, while that in the Philippines seems to present characteristics of both Denmark and Travancore-Cochin. The differences have to do primarily with the relative proportion of reactions in the range from 4 to about 12 mm. In Denmark, as previously noted for the adult population, the 5-TU reactions are concentrated into two distinct groups, interpreted as the negatives and the positives, which are separated from each other by a point of very low frequency (0.4 per cent) at 6 to 7 mm. In the Philippines, there is an indication that the 5-TU distribution is also composed of two groups, although the point of separation seems to be at 12 to 13 mm. instead of at 6 to 7 mm. Relatively fewer of the negative reactions measured 0 to 1 mm. in Filipino children compared with those in Danish children, and a far greater proportion of them measured between 2 and 12 mm. Finally, the 5-TU distribution for Travancore-Cochin does not give even a suggestion of a point of separation between negative and positive reactions because of the large numbers of small reactions.

One might have been tempted to conclude from these findings that the differences in frequencies of small reactions to the 5-TU test in these three widely separated parts of the

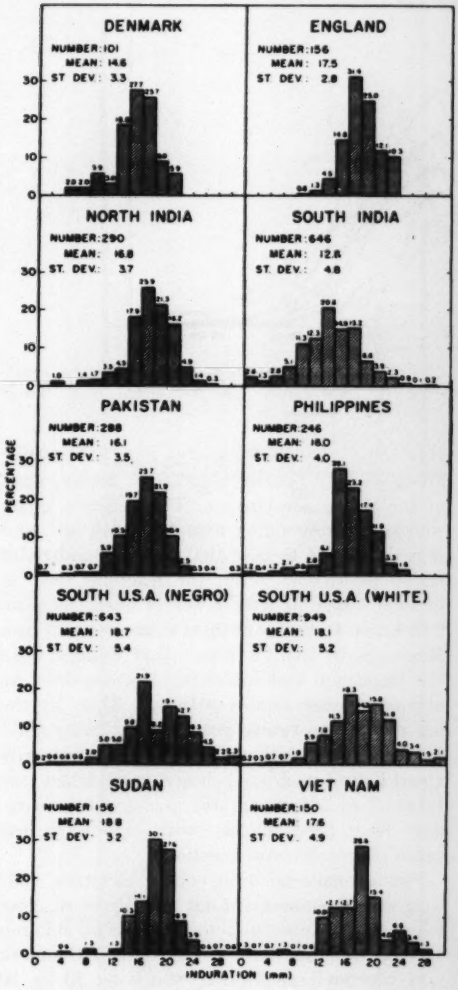

FIG. 5. Distributions of sizes of reactions to 5 TU of PPD for patients in tuberculosis hospitals in various countries. (*Reproduced with permission from WHO Tuberculosis Research Office, Bull. World Health Organ., 1955, 12, 63.*)

world represent racial differences, or differences related to nutritional factors, or even possibly differences in the antigenic properties of tubercle bacilli. But that conclusion cannot stand in the face of the finding that persons known to be infected with tubercle bacilli, namely tuberculous patients, in different parts of the world show a strikingly similar response to the 5-TU test, despite obvious differences in race, nutritional status, and many other factors (cf. figures 5 and 6).

One clue to the interpretation of these findings lies in the differences in the sensitivity of the groups of children to the 100-TU test. Most of the Danish children who were given the 100-TU test (because their reactions to 5 TU were less than 5 mm.) still had reactions of less than 5 mm. In contrast, most of the children in the Philippines had reactions of more than 5 mm. to 100 TU; and in Travancore-Cochin virtually every single child reacted to the strong dose. It is readily apparent that there is very little strong-dose sensitivity in Denmark, but it is highly prevalent in the Philippines and even more highly prevalent in Travancore-Cochin.

The increased proportion of small (2 to 12 mm.) reactions to the 5-TU test seen in the Philippines and in Travancore-Cochin, as compared with that in Denmark, reflects the increased prevalence of sensitivity that is brought out by a strong dose of tuberculin. This is the kind of sensitivity which is interpreted as caused not by *M. tuberculosis* but by some other microorganism. In other words, the present writers believe that most of the "excess" of small reactions to 5 TU and nearly all of the reactions of any size to 100 TU found in the Philippines, Travancore-Cochin, some parts of the United States, and in many other regions of the world, are cross reactions to tuberculin caused by sensitization by infection with other organisms.

Material collected in the Somalilands (72) offers further support of that interpretation by showing that strong-dose sensitivity may appear at a much earlier age than low-dose sensitivity. Results are given in figure 10 for a group of tuberculous patients who were tested with 5 TU and for school children and general populations tested with 5 and 100 TU. The 100-TU test was given to all persons who reacted with less than 10 mm. of induration to 5 TU. For the youngest children, almost all of the reactions to 5 TU were measured as zero or only a millimeter or two; practically none was large enough to be called positive. In the next age group, four to seven years, a few had large reactions which form a group centered around 16 mm.

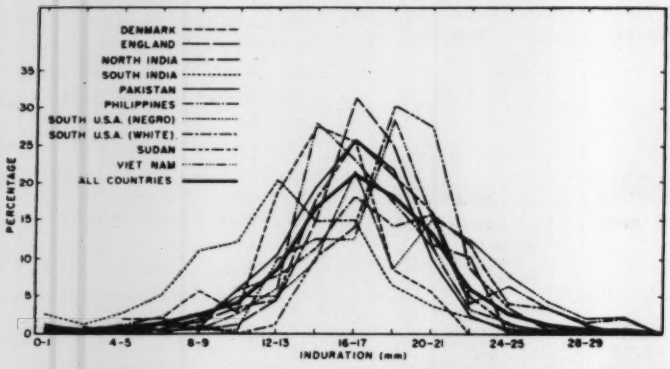

FIG. 6. Frequency curves of sizes of reactions to 5 TU of PPD for patients in tuberculosis hospitals in various countries. *(Reproduced with permission from WHO Tuberculosis Research Office, Bull. World Health Organ., 1955, 12, 63)*

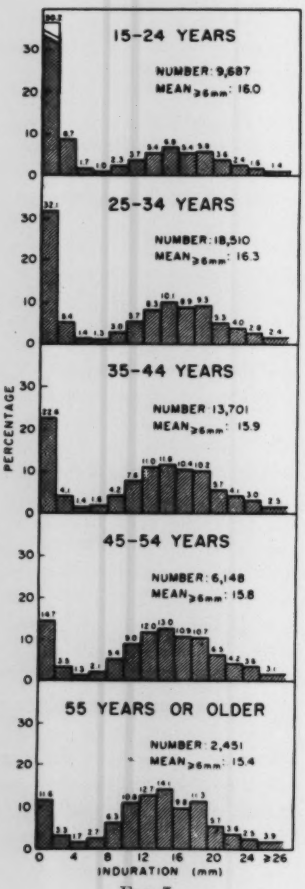

FIG. 7

Increasing numbers of children join that positive group as they become older; and, finally, most of the adults are positive. The negative group shows a corresponding reduction with age; and it is significant to note that, as the numbers of negatives become lower, the reactions tend to become larger in size: fewer of them measure 0 to 1 mm. and more of them measure 4 to 5 mm. Reactions to 100 TU reflect that change: both the frequency and intensity of strong-dose reactions increase rapidly with age. Thus, by the age of eight to twelve years, almost every child has become sensitive to tuberculin, but only about half of them are believed to have had true tuberculous infections; the remainder presumably have been infected with organisms that cause tuberculin cross reactions.

Similar material from other countries (68) suggests an estimated total prevalence of cross reactions to tuberculin among school children ranging from less than 10 per cent in Denmark and the northern United States to 20 or 30 per cent in England and Mexico, around 50 per cent in Ethiopia and in the southeastern United States, and more than 90 per cent in the Philippines and the Sudan. In discussing the serious problem disclosed by their findings, Palmer and his associates in the WHO Tuberculosis Research Office wrote, in 1955, that

> . . . with the tuberculin products in use today, we are unable to distinguish the smaller

FIG. 7. Distributions of sizes of reactions to 10 TU of PPD (RT), by age, for a general population in Denmark. *(Reproduced with permission from the Danish Tuberculosis Index, Bull. World Health Organ., 1955, 12, 261.)*

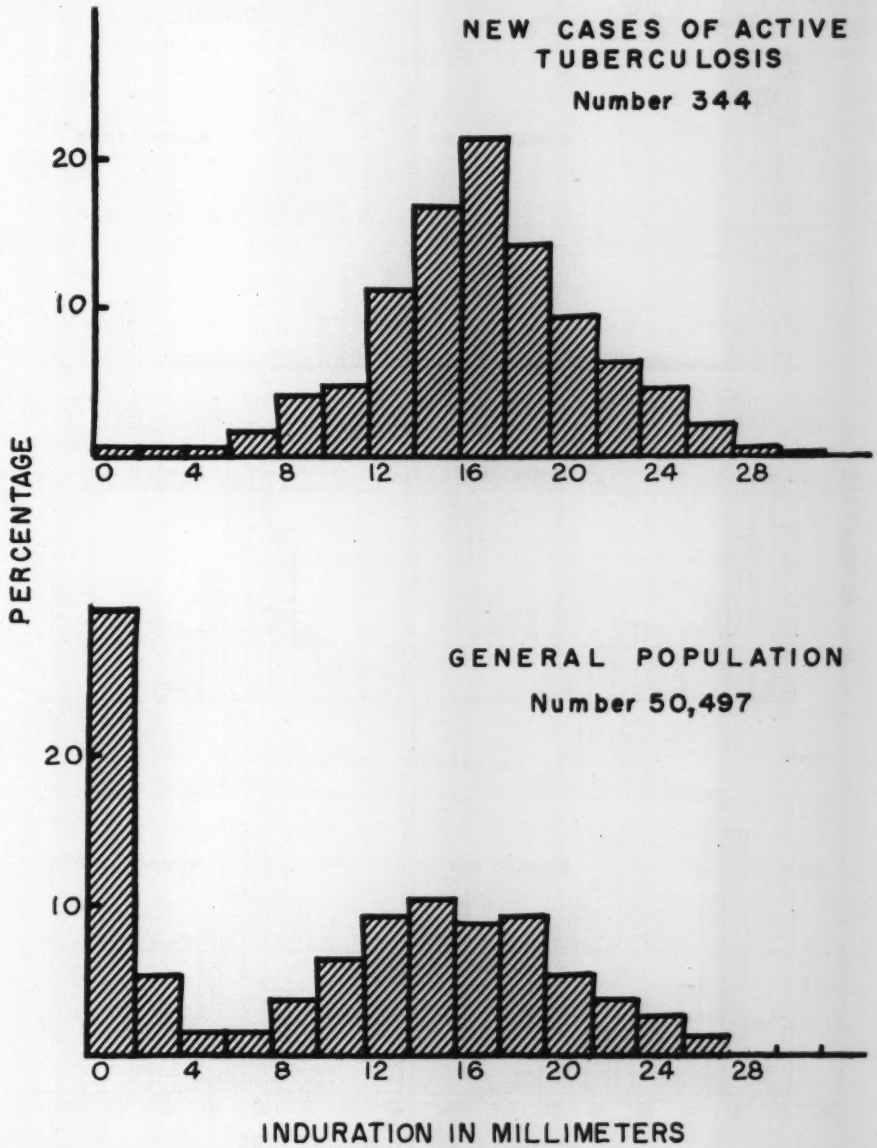

FIG. 8. Distributions of sizes of reactions to 10 TU of PPD (RT) for a general population in Denmark and for new cases of tuberculosis found in that population. (*Adapted from the Danish Tuberculosis Index, Bull. World Health Organ., 1955, 12, 261*)

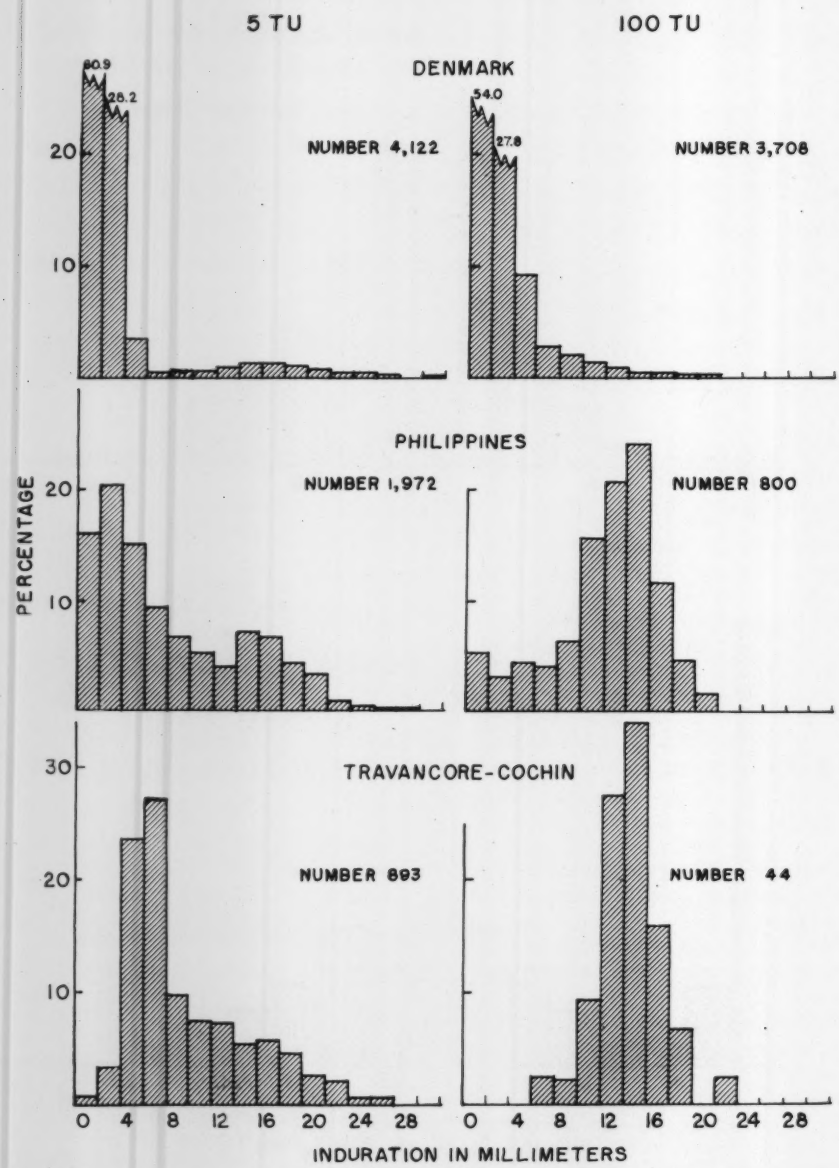

FIG. 9. Distributions of sizes of reactions to 5 TU or 100 TU of PPD (RT) for school children in three different countries. (*Reproduced with permission from WHO Tuberculosis Research Office, Bull. World Health Organ., 1955, 12, 63 and 101*)

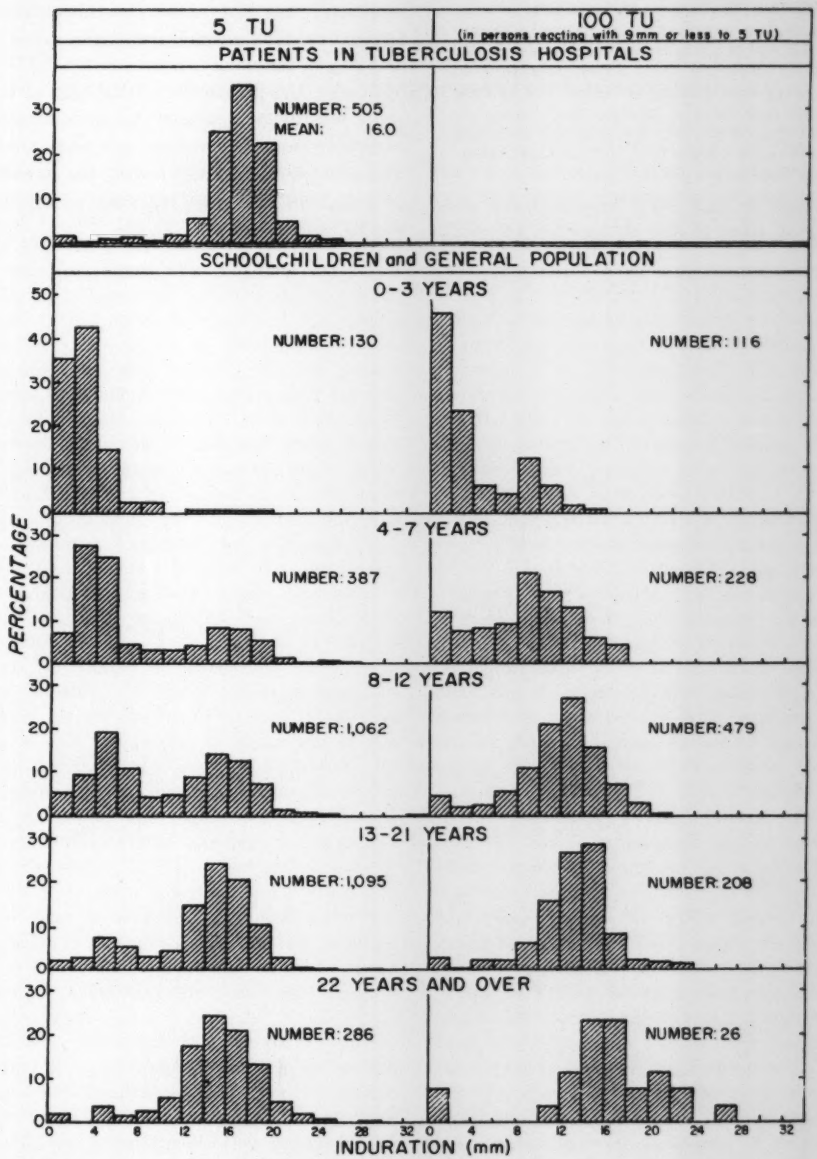

FIG. 10. Distributions of sizes of reactions to 5 TU of PPD (RT) for tuberculous patients, and to 5 TU or 100 TU for a general population by age, in the Somalilands. (*Reproduced from mimeographed material from the WHO Tuberculosis Research Office, 1956*)

specific from the larger non-specific reactions. Most of the overlapping apparently occurs in the range of 6 mm to about 12 mm with the 5 TU intradermal test. Thus, reactions in this range may belong to either the specific or the non-specific group: they must be considered 'doubtful,' not because they are equivocal in character, but because their interpretation is doubtful (68).

Although it had been suspected for some years that most of the strong-dose tuberculin sensitivity was not caused by tuberculous infection, by 1955 suspicion was also being cast on many of the small reactions to the 5-TU test. Moreover, the prevalence of this kind of tuberculin sensitivity appeared to show enormous variations from one geographic area to another. The next step in further testing the hypothesis of nonspecific or cross reactions to tuberculin was to find the causative agent.

CHAPTER VIII

SEARCH FOR THE CAUSE OF TUBERCULIN CROSS REACTIONS

The nature, as well as the variations in prevalence, of the tuberculin sensitivity interpreted as cross reactions among apparently normal, healthy populations in various parts of the world led logically to several inferences about the causative agent. To sensitize very large numbers of persons, the causative agent(s) would have to be not only abundant, but also readily accessible. To sensitize to the human type of tuberculin, the agent would have to possess antigenic components in common with tubercle bacilli of human origin, so it is probably a Mycobacterium. And to sensitize without producing evident signs of disease the agent would have to be essentially nonpathogenic for most people, even though, in some circumstances, it might produce recognizable clinical disease.

The avian tubercle bacillus was, for several reasons, among the first to be considered a possible source of tuberculin cross reactions in human beings. It had long been recognized as one of the sources of nonspecific (cross) sensitivity in cattle. In human beings, as in cattle, it rarely causes disease. The early studies of Mariette and Fenger (32) in Minnesota as well as those of McCarter and her associates (46) in Wisconsin had shown that sensitivity to avian tuberculin was highly prevalent among residents of those states. The more recent studies of other workers (73, 74) showed that sensitivity to avian tuberculin is also highly prevalent in several other parts of the world; and, moreover, that avian PPD appears to be an effective antigen for identifying persons whose reactions to human tuberculin are considered to be cross reactions.

Infection with avian tubercle bacilli cannot, however, be incriminated as the sole or perhaps even the most frequent cause of cross reactions to human tuberculin for the reason that antigens prepared from a variety of other types of acid-fast organisms are equally effective in identifying persons with that kind of reaction. In 1957, Edwards and Krohn (74) reported results of studies conducted among groups of persons in India and in the Philippines, where comparative skin tests were given with human PPD, avian PPD, and PPD antigens prepared from *Mycobacterium balnei*, *Nocardia intracellularis*,[11] and two unidentified chromogenic mycobacteria isolated in India from sputum of tuberculosis suspects. *Mycobacterium balnei* is the "swimming pool organism," which was isolated in Sweden by Lindell and Nordén (77) from ulcerative skin lesions in youngsters with histories of abrasions of their legs and arms caused by contact with the walls of a swimming pool. The organism reported as *Nocardia intracellularis*[11] by Cuttino and McCabe (78) was isolated in North Carolina from a patient with granulomatous disease; and the antigen prepared from it is referred to as the "Cuttino" antigen or PPD-C.

Results of skin tests with these various antigens revealed that many persons with small reactions to the 5-TU dose of human PPD, i.e., reactions ranging from 3 to 12 mm. in size, were equally or more sensitive to approximately equivalent doses of the PPD antigens prepared from the two chromogenic mycobacteria from India, the avian, the *M. balnei*, and the Cuttino organisms. In contrast, most persons with reactions of 12 mm. or more to human tuberculin had clearly smaller reactions to the other antigens. "Thus," the writers concluded (74), "the low-grade tuberculin sensitivity to purified human tuberculin seems to reflect a response to an antigenic factor common to various types of acid-fast organisms and not a specific factor in human tuberculin, whereas the higher degrees of sensitivity to purified tuberculin appear to represent a specific response to tuberculous infection."

Even in Koch's time the possibility of some common denominator in two mycobacterial diseases, tuberculosis and leprosy, was recognized, and it is still being pursued. Lowe and McNulty (79) have presented both supporting and conflicting evidence from various parts of the world that leprosy may be a source of tuberculin sensitivity. Many other acid-fast organisms capable of producing sensitivity in man to mammalian tuberculin have now been identified;

[11] This organism has recently been reported by other workers as probably a Mycobacterium rather than a true Nocardia (75, 76).

and the number increases almost daily. In 1930, Beaven (80) isolated an organism he called *Mycobacterium Ryan* and, with a "tuberculin" prepared from it, was able to elicit reactions in both animals and humans. The recognition in the early 1930's that bovine, avian, and timothy grass bacilli could produce cross reactions to human tuberculin aroused further interest in acid-fast organisms. By 1946, Hauduroy (81) was able to list more than 100 "bacilles paratuberculeux" and detail their morphologic and cultural characteristics as well as their pathogenicity for various laboratory animals. MacCallum and his associates (82) reported the *Mycobacterium ulcerans* in 1948; Buhler and Pollak (83), the "yellow bacillus" in 1953; Linell and Nordén (77), the *Mycobacterium balnei* in 1954; Crow and his associates (84), the "Battey" Mycobacterium in 1957. And so the roster grows. Runyon (85) is now collecting these organisms which he terms "anonymous" mycobacteria and already has more than 400 strains which he classifies into four broad groups: photochromogens, skotochromogens, nonphotochromogens, and rapid growers.

Because of the high prevalence in the southeastern part of the United States of reactions believed to be cross reactions, Palmer and his co-workers reasoned that the causative agent, which must be widespread and infecting a great many persons in that region, might cause an occasional case of clinical disease. The fungus Histoplasma, which is highly prevalent in the east-central part of the country, infects large numbers of persons and sensitizes them to histoplasmin but seldom causes clinical disease; so, by analogy, an unidentified acid-fast organism might be found among patients in the Southeast with clinical disease of unknown etiology. In discussing the matter in 1952 with Dr. Rufus Payne, who at that time was Superintendent of the Battey State Tuberculosis Hospital in Rome, Georgia, Palmer was told that an "atypical acid-fast organism" had been isolated repeatedly from sputum of more than thirty patients at Battey Hospital with clinical disease resembling tuberculosis. He was given several cultures of the organism which he took to the State Serum Institute in Copenhagen for the purpose of having a PPD antigen prepared by the same procedure as that used for the preparation of the Danish PPD tuberculin. Although an antigen was not made from it at that time, cultures of presumably the same type of organism isolated more recently from patients at Battey Hospital were obtained from Dr. Raymond Corpe in 1956. These cultures were given to Dr. Florence Seibert at the Henry Phipps Institute in Philadelphia, and a PPD antigen was prepared under her direction by Affronti (86), using the same technique as for the preparation of PPD-S, the international standard PPD

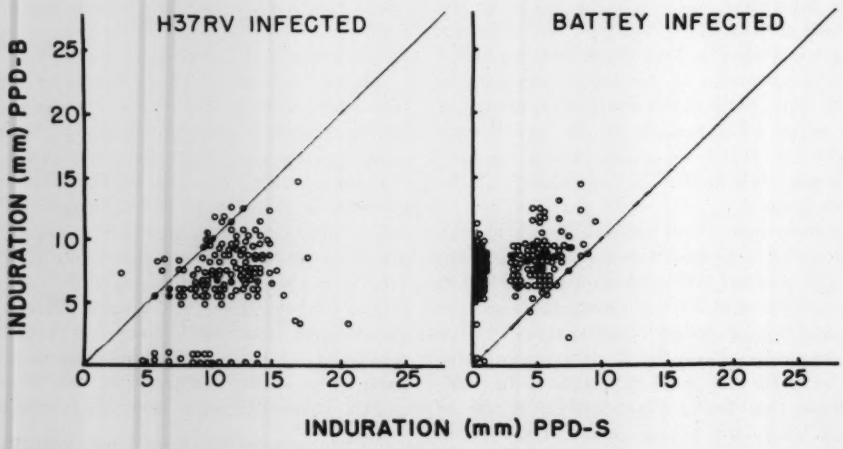

FIG. 11. Correlation of sizes of reactions to 0.0005 mg. of PPD-S and PPD-B in guinea pigs. (*Reproduced with permission from Palmer et al., J. Pediat., 1959, 55,* 413)

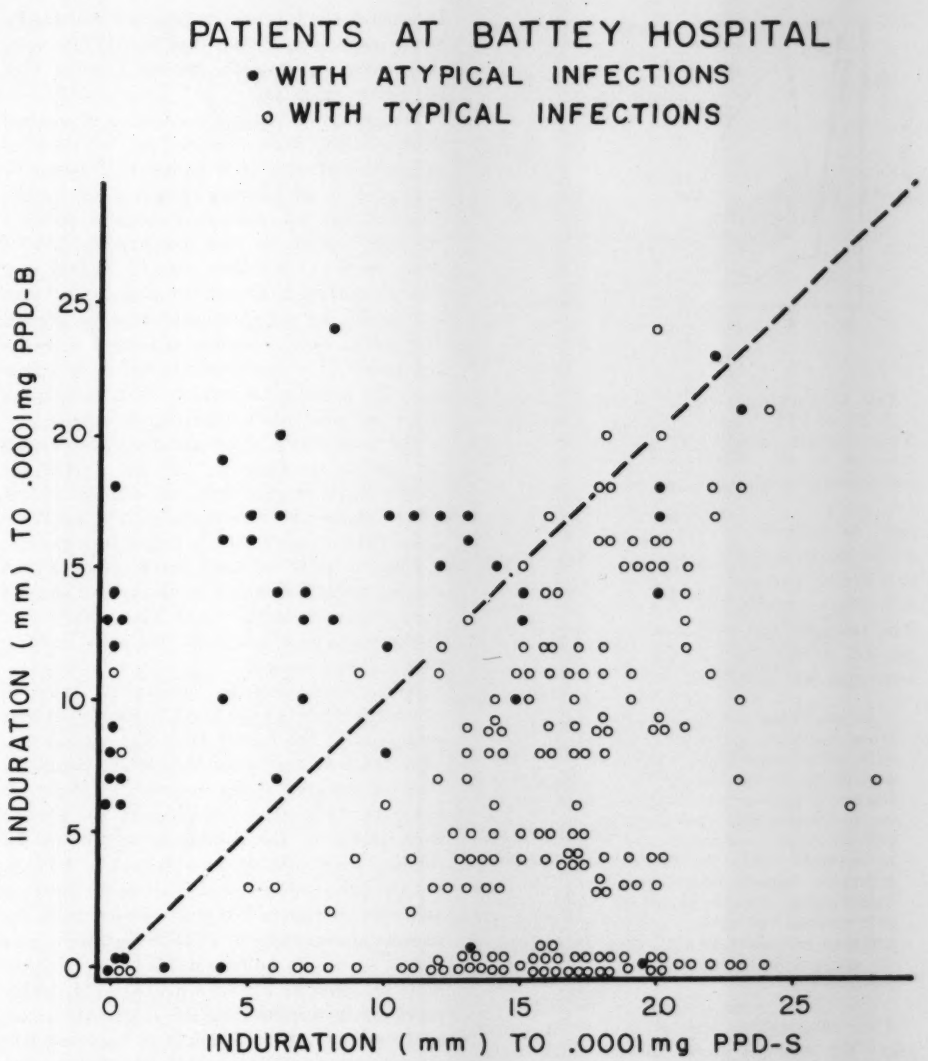

FIG. 12. Correlation of sizes of reactions to 5 TU of PPD-S and 0.0001 mg. of PPD-B for patients in a tuberculosis hospital in Georgia. (*Reproduced with permission from Edwards et al., Acta tuberc. scandinav., 1959, Supplement No. 47, p. 77*)

tuberculin. Antigens have also been made for Palmer and his associates in the Public Health Service from a variety of other organisms, with preference given to acid-fast organisms isolated in areas where tuberculin cross sensitivity is highly prevalent, and organisms occasionally isolated from patients with clinical disease resembling tuberculosis.

Results of comparative tests with some of the new antigens and PPD-S have recently been reported by the Public Health Service group (87–89). Studies have been conducted in guinea

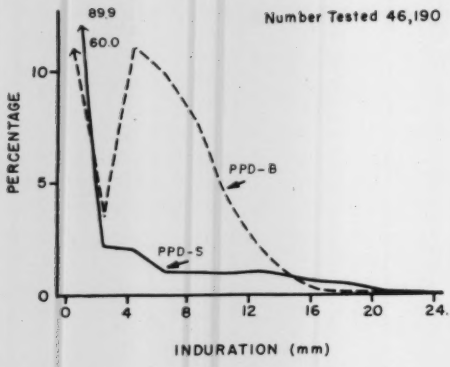

FIG. 13. Frequency curves of sizes of reactions to 5 TU of PPD-S and 0.0001 mg. of PPD-B for Navy recruits. (*Reproduced with permission from Edwards et al., Acta tuberc. scandinav., 1959, Supplement No. 47, p. 77*)

pigs, in patients in tuberculosis hospitals, in school children, and in general population groups in different parts of the United States, as well as in Navy recruits from all parts of the country. The rationale for comparative testing was expressed in the 1959 report by Edwards and associates (88) as follows:

> In searching for the cause of tuberculin cross reactions, we reasoned that if infection with other organisms results in low-grade sensitivity to tuberculin, then comparative tests with appropriate antigens should enable us to distinguish specific tuberculin sensitivity from cross reactions. We knew that in suitable doses the homologous antigen generally causes larger reactions than a heterologous antigen. Moreover, comparative skin testing had already proved of practical value to veterinarians attacking the problem of 'nonspecific' or cross reactions to tuberculin in cattle.

For comparative testing studies in guinea pigs, the animals were infected with either virulent tubercle bacilli (H37Rv) or the Battey organism, and were tested with 25 TU (0.0005 mg.) of PPD-S and with the same dose of PPD prepared from the Battey strain (PPD-B). It may be seen in figure 11 that, with only a few exceptions, it was possible to differentiate, by the comparative sizes of the reactions to the two antigens, the animals infected with H37Rv from those infected with the Battey organism. In general, the tuberculous pigs had a markedly larger reaction to PPD-S than to PPD-B, while the reverse was true for animals infected with the Battey organism.

The results of comparative testing of patients at the Battey State Hospital parallel those of the animal studies. It is indicated in figure 12 that most of the patients harboring the Battey organism can be distinguished from patients with tuberculosis by the comparative sizes of their reactions to 0.0001 mg. of PPD-B and PPD-S. Although identification from the skin test results did not correspond with the clinical diagnosis in every case, the agreement was of a high order. Thus, in humans as well as in guinea pigs, the homologous antigen, as a rule, gives larger reactions than a heterologous antigen.

The possibilities of comparative skin testing are further demonstrated by the results for 46,000 Navy recruits from all sections of the United States who were tested with both PPD-S and PPD-B (figure 13). As might be expected, a large majority of these young men had no reaction to PPD-S, and only 10 per cent showed some reaction. On the other hand, nearly half of the recruits (40 per cent) had some reaction to the Battey antigen.

When considering the geographic areas in which the recruits have lived, it was found that sensitivity to the Battey PPD was a great deal more frequent among residents of the Southeast than of the rest of the country, as shown in figure 14. More than two-thirds of the recruits from states in the southeastern part of the country react with at least 4 mm. to PPD-B, but throughout wide stretches across the northern and western regions less than one-fourth of the recruits are sensitive to PPD-B. Can the source of this sensitivity be responsible for some of the cross reactions to PPD-S which are also highly prevalent in recruits from the southeastern part of the country? If so, it might be expected that the reaction to PPD-S would be smaller than the reaction to PPD-B in the comparative test.

Support of that hypothesis is offered by the data in figure 15. Recruits from the North and West and those from the Southeast who had "positive" reactions (6 or more mm.) to PPD-S have been divided according to the size of their reactions to PPD-B into the following three groups: those whose reaction to PPD-B was larger than to PPD-S (cross-hatched area); those

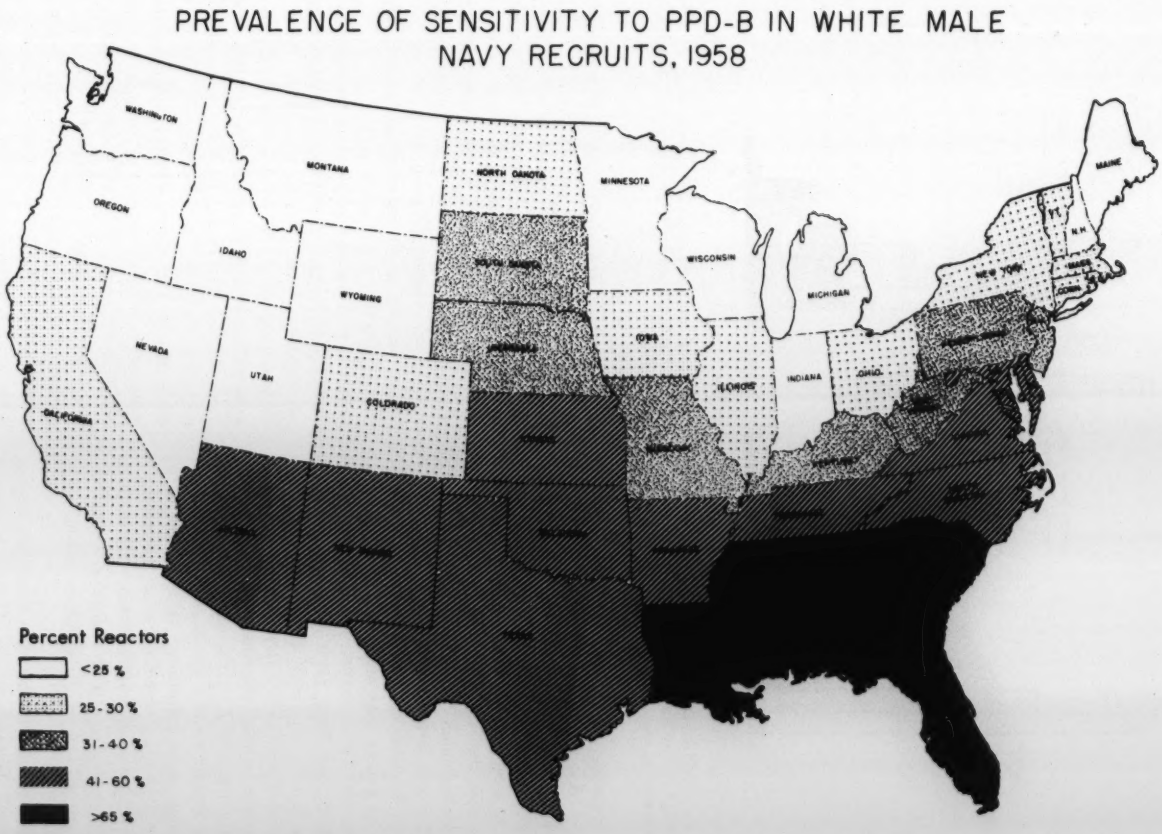

FIG. 14. Percentages of 46,000 Navy recruits with reactions of 4 or more mm. to 0.0001 mg. of PPD-B, by state of residence.

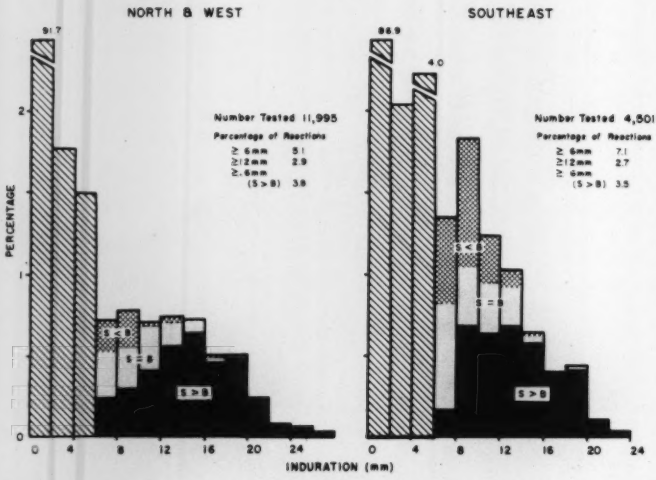

FIG. 15. Distributions of sizes of reactions to 5 TU of PPD-S, and relative sizes of reactions to 0.0001 mg. of PPD-B, for Navy recruits from different parts of the United States. (*Reproduced with permission from Edwards et al., Acta tuberc. scandinav., 1959, Supplement No. 47, p. 77*)

whose reactions to the two antigens were the same size or within 1 mm. of each other (lightly shaded area); and those whose reaction to PPD-S was larger than to PPD-B (darkly shaded area). It may be seen that a much higher proportion of recruits from the Southeast than from the North and West had small "positive" reactions to 5 TU of PPD-S and larger reactions to PPD-B. It is reasoned, by analogy with the findings of the studies of guinea pigs and the Battey Hospital patients, that these recruits have cross reactions to PPD-S and have been infected, not with tubercle bacilli, but with the Battey organism or one closely related to it. By the same reasoning, the recruits whose reactions to PPD-S were larger than to PPD-B (the darkly shaded area in each figure) can be considered to have had true tuberculous infections. It is to be noted that the frequency distribution of this group is similar in both geographic areas and bears a strong resemblance to the distribution of reactions to PPD-S of tuberculous patients (*cf.* figure 5).

The prevalence of tuberculin sensitivity among young men throughout the United States is mapped in figure 16, based on results for nearly 70,000 Navy recruits, seventeen to twenty-one years of age, who were tested during the year 1958 with the standard 5-TU dose of PPD-S. This group is considered fairly representative of the young white male population of the entire country. All those with reactions of 8 mm. or more were classified as tuberculin reactors and were distributed according to their states of residence. A criterion of 8 mm. rather than 6 mm. was used in order to reduce somewhat the number of persons believed to have cross reactions among those classified as reactors, particularly in the southeastern region.

Just over 5 per cent of all of the recruits were classified as tuberculin reactors, the percentages for separate states ranging from 2 to 10. The highest rates are found in the Southwest and in a band running through Kentucky and West Virginia into Maryland. In the Southeast, the rates are variable and undoubtedly reflect, to some extent, the effect of cross reactions. For large areas of the country extending from the Great Lakes to the Pacific Ocean, the rates are well below 4 per cent.

Since the frequency of tuberculin reactors among persons who are now twenty years of age is the cumulative result of infections which have occurred at sometime during the last twenty years, the total prevalence of 5 per cent found in these young men corresponds to an average infection rate of 2 per 1,000 per year from 1938

PREVALENCE OF TUBERCULIN SENSITIVITY IN WHITE MALE NAVY RECRUITS, 1958
(66,400 TESTS WITH 5 TU, PPD-S)

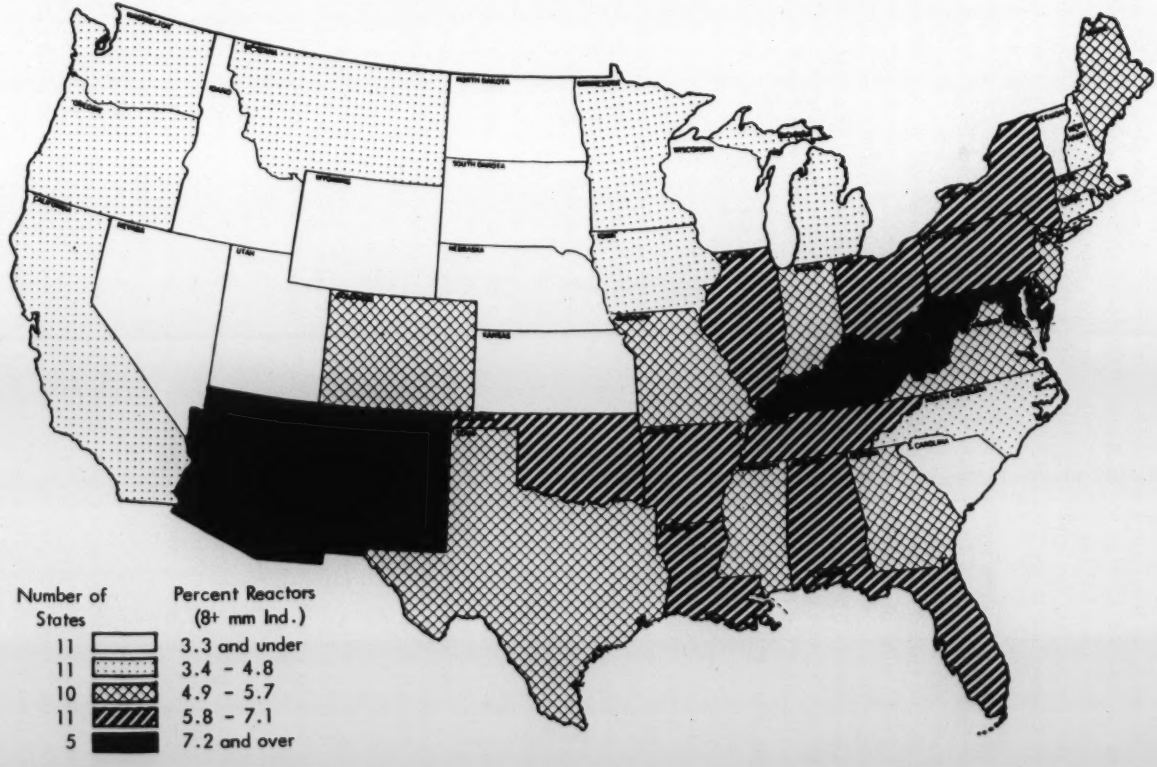

FIG. 16. Percentages of 70,000 Navy recruits with reactions of 8 or more mm. to 5 TU of PPD-S, by state of residence.

to 1959. However, as there are many indications that the infection rate has not been constant during these years but has been declining rapidly, the infection rate throughout large parts of the country must now be less than 1 per 1,000 per year.

The consequences of these and other studies now in progress can be expected to open the way for programs aimed at the final eradication of tuberculosis. It is clear that tuberculous infection is not the only cause of tuberculin sensitivity. The evidence from both experimental animals and human patients with clinical disease shows that tuberculin sensitivity may be caused by infection with other types of mycobacteria, just as it may be caused by virulent tuberculous infection. The same kind of evidence applies to the interpretation of tuberculin sensitivity in healthy persons. With the development of a simple, practicable way to distinguish persons who have had true tuberculous infections from those with other mycobacterial infections, the medical profession will be able to focus attention on the small (and diminishing) numbers infected with tubercle bacilli who are at high risk of developing tuberculous disease. Effective antimicrobial agents are now available for treatment of clinical disease. And with the present hopeful indications of effective prophylaxis, even the most conservative can look toward the day when tuberculosis will rarely be found except in the textbooks.

CHAPTER IX

OUTLOOK FOR THE FUTURE

For the tuberculin test to have finally been revealed as akin not to one bacillus but to many may be disconcerting from one point of view but reassuring from another. There may be nostalgia for the days when all tuberculin reactions meant tuberculous infection, and no nonsense! On the other hand, the imagination is now stirred by the promise that, with more reliable means for identifying persons who have had tuberculous infections, more effective steps can be taken for the eradication of the disease.

Seventy years ago, Koch's announcement that he had discovered a curative agent against tuberculosis was instantly acclaimed by both the lay and professional press; but praise soon turned to criticism as the failure of tuberculin as a therapeutic agent became evident. The potentialities of tuberculin for detecting tuberculous infection were quickly recognized, however, particularly by the veterinary profession. Although enthusiasm was dampened some years later when an increasing proportion of the cattle, slaughtered because of tuberculin reactions, showed no signs of tuberculosis, faith in the tuberculin test was restored when the existence of nonspecific or "false positive" reactions in cattle was established and practical means of identifying the "true positive" were developed. Meanwhile, in human medicine the reliability of the tuberculin test as a diagnostic tool remained unquestioned. As its use was extended from the clinic to general population groups, however, difficulties arose in connection with administration and dosage, and with the problem of pulmonary calcifications in nonreactors. As research on these and other questions progressed, evidence gradually emerged that all reactions to tuberculin were not necessarily indicative of tuberculous infection. The quantitative aspects of sensitivity took on new significance. Today, results of laboratory, clinical, and large-scale population studies have established the existence in nature of a variety of acid-fast organisms capable of sensitizing man to human tuberculin.

The current torrent of newly recognized mycobacteria augurs new stories as yet untold. There is much to sort out and much to learn about the newcomers. Which ones are benign? Which ones produce disease? Where in the world are they, and how prevalent are they?

Recent developments in research on the group of miseries variously called the grippe, "a virus," or old-fashioned "flu" afford a striking parallel. According to Kilbourne (90), within the past five years ". . . some 25 new respiratory viruses have been isolated and recognized for the first time." In describing one of the new groups known as the adenoviruses, he writes:

> As with all the other newly recognized viruses, the importance of the adenoviruses in human respiratory disease is largely unknown and remains the subject of intensive investigation. Studies have shown that infection with certain of these viruses in the first five years of life is exceedingly common, although the severity of the *disease* associated with such infections is not known. . . .
>
> . . . revival of tissue culture for virus research has led very recently to the recognition of other viruses distinct from the influenza viruses and adenoviruses. Certain of these viruses—the hemadsorption, or "para-influenza" viruses—appear to be biologically related to the influenza viruses. . . .
>
> Preliminary information seems to indicate that these viruses, too, are common causes of infection in man. The CA virus, or "croup-associated virus," as well as the CCA virus (chimpanzee coryza agent), are similarly associated with some cases of infantile croup. The names of these two latter agents illustrate the confusing and erratic nomenclature employed with human viruses.

Kilbourne concludes by stressing that one of the first jobs is to define the natural history and virulence of these viruses in the population at large.

Substitute the word mycobacteria for respiratory viruses; photochromogenic mycobacteria for adenoviruses; yellow bacillus, Battey organism and rapid grower for hemadsorption virus, CA, and CCA. There is a familiar ring! Even the implication in Kilbourne's conclusion has its parallel: we know very little about the natural history and virulence of these mycobacteria; very little of their prevalence and distribution in different regions of the world, their source and reservoirs of infection, and their sensitizing properties.

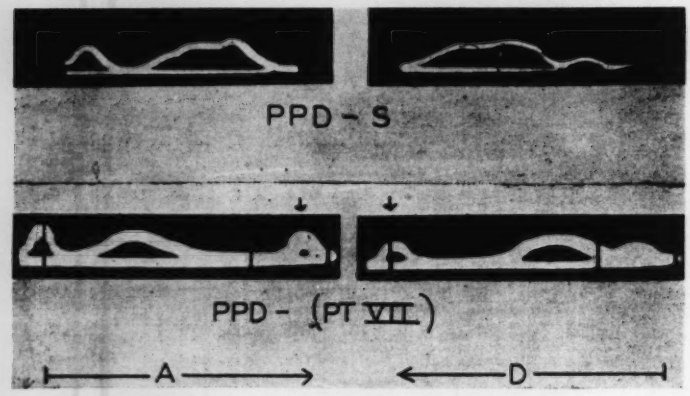

FIG. 17. Electrophoretic diagrams of two PPD products: ammonium sulfate precipitated PPD-S, and trichloracetic acid precipitated PPD-(PT VII). (*Reproduced with permission from Seibert and Dufour, Am. Rev. Tuberc., 1948, 58, 363*)

The rapid expansion of our knowledge of these mycobacteria and those yet to be discovered has been, and will continue to be, facilitated by adaptation of old techniques and the development of new ones.

The quantitative statistical technique, which was adapted for epidemiologic studies of tuberculin sensitivity and has revealed a complex of constituent parts formerly thought to be an indivisible entity, has an interesting counterpart in the electrophoretic technique used in the fractionation of the complex tuberculin and identification of many of its constituent parts.

As the frequency histogram provides information about the purity of the tuberculin sensitivity in a population, so the electrophoretic diagram provides information about the purity of the substance in question. For example, the electrophoretic diagrams reproduced in figure 17 are from a report by Seibert and Dufour (91), published in 1948, on studies of PPD. The figure compares the standard tuberculin product PPD-S with an earlier PPD product which was very similar to PPD-S but differed in having an extra, fast component (indicated by the arrow) identified as nucleic acid. The first peak on the left of the ascending limb for both products represents polysaccharide; and the larger, more irregular, peak represents protein. "As chemical purity is the goal in any such isolation," Seibert wrote, "the ability to remove most of the . . . nucleic acid and . . . polysaccharide, which are inert substances in the tuberculin reaction, must be considered an advanced step in the purification. The resulting product, which is practically nothing but protein, can now be fractionated into its component proteins. . . ."[12]

Tuberculin skin testing studies appear now to have reached about the same stage of development. By means of the frequency histogram of sizes of reactions to 5 TU of PPD-S, various peaks can be identified which are interpreted as representing different "impurities" in the response to tuberculin. These impurities are the reactions ascribed to infection with related mycobacteria. As an example, frequency histograms are shown in figure 18 of sizes of reactions to the 5-TU test for general population groups in two parts of Africa, Libya and the Sudan (92, 93). As indicated by the schematic curves, the histogram for the group tested in Libya is interpreted as having only two components: negative reactions and "pure" specific tuberculous reactions. The histogram for the group tested in the Sudan, on the other hand, is interpreted as having those same two components and, in addition, a third one which represents cross reactions. The problem, clearly, is to "fractionate" out the cross reactions. Here, the comparative test with certain

[12] It might be said here, parenthetically, that the hitherto unsuccessful efforts to purify tuberculin have not been wholly disadvantageous. It is, ironically, the "impurities" in PPD that are responsible for the cross reactions to tuberculin which might otherwise have remained undiscovered.

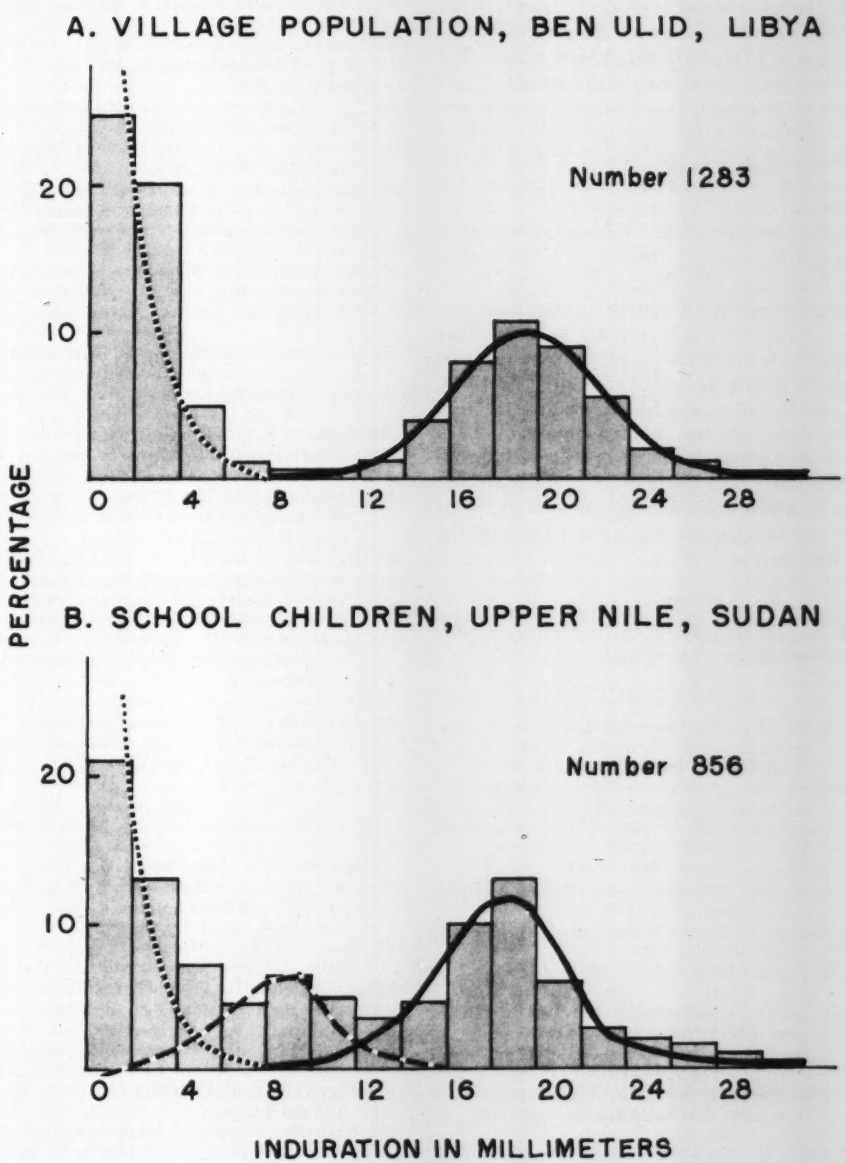

FIG. 18. Distributions of sizes of reactions to 5 TU of PPD (RT), with schematic curves showing component segments, for two general population groups. (*Adapted from mimeographed material from the WHO Tuberculosis Research Office, 1955 and 1956*)

of the antigens recently prepared from various kinds of so-called "atypical" mycobacteria provides a way to separate out at least some of the cross reactions. Since most of the antigens presently available, including PPD-S, appear to act as group-specific rather than type-specific, further fractionation within the various groups should be possible as appropriate new antigens are developed.

Further stories of the tuberculin test thus remain to be told; and they will no doubt deal with the variety of causes, or at least the major causes, of tuberculin sensitivity in human populations. These stories will probably be followed by others which will relate the means by which true tuberculous infection can be accurately identified. For, as Hastings and his associates (15) said twenty-five years ago, in discussing the problem of distinguishing between specific and nonspecific tuberculin reactions, ". . . there are few areas in which great numbers of tests are being made in which the problem is not deemed worthy of consideration . . ."

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